The Design and Synthesis of Acylated Enamino Esters as Potential Inhibitors of Serine Proteases

A Thesis

presented for the degree of

Doctor of Philosophy in Chemistry

at the

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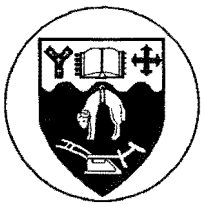
by

Jane M. Taylor



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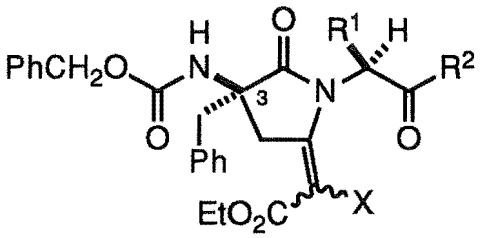
ABSTRACT

This thesis examines the synthesis of bromo acylated enamino esters, of the type (**4.01**), and protio acylated enamino esters, of the type (**4.02**), designed as a new class of potential mechanism-based inactivators and alternate substrate inhibitors, respectively, of chymotrypsin.



4.01 X = Br
4.02 X = H

Chapter 1 describes the synthesis of succinic-, glutaric- and phthalic-based, bromo and chloro enollactones (**1.11-1.15, 1.17**) via a new reaction involving halo enol-lactonization of keto acid phosphoranes (**1.06-1.10**). The phthalic bromo enollactones (**1.17**) are also synthesized via the reaction of $Ph_3P{=}CBrCO_2Et$ with 4,5-dichlorophthalic anhydride. The reactions are extensions of the SCOOPY and Wittig anhydride carbonyl olefination reactions.

Chapter 2 describes the synthesis of simple succinimide- and phthalimide-based protio and chloro enamino esters (**2.09, 2.63-2.75, 2.93, 2.95, 2.98**) via a versatile new reaction in which an enollactone (**2.33, 1.11, 2.115**) and amine react to form an isolable keto-amide (**2.37-2.47, 2.94, 2.96, 2.116**) and/or hydroxy lactam (**2.48-2.50, 2.97**) intermediate. Subsequent elimination of H_2O on heating gives the enamino ester via an overall insertion process.

Chapter 3 describes the synthesis of simple succinimide-based protio and bromo enamino esters (**2.66, 2.71, 3.02-3.03**) from the reaction of β-keto ester (**3.01**), via an isolable enamine intermediate (**3.07-3.10**). The synthesis of a 3-substituted enamino ester (**3.04**) via the insertion and β-keto ester routes is also described.

Chapter 4 describes the synthesis of the target 3,3-disubstituted enamino esters (**4.01-4.07**) via the insertion reaction and a $TiCl_4$ mediated β-keto ester reaction. The benzyl group at position 3, required for recognition by chymotrypsin, is introduced stereoselectively using methodology developed largely by Seebach and coworkers for the asymmetric synthesis of α,α-disubstituted amino acids. By changing this residue other serine proteases can be targeted. The proposed inhibitors (**4.01-4.07**) are designed to be incorporated into an oligopeptide having optimum interaction with the target enzyme. Preliminary testing of enamino esters and enollactones (**2.71, 3.04, 4.01-4.02, 4.03, 4.06, 4.42, 4.43**) for chymotrypsin inhibition is also described.

Chapter 5 examines trends in the ^{13}C NMR, ^{31}P NMR and FAB mass spectra of keto acid and keto ester phosphoranes (**4.15, 4.26, 4.40-4.41, 5.04-5.06**); key intermediates to enamino esters and enollactones (*eg* **4.01-4.06, 4.42, 4.43**).

ACKNOWLEDGEMENTS

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INTRODUCTION

SECTION I.1

PROTEASE ENZYMES

Proteases constitute a large family of enzymes which catalyze the hydrolytic cleavage of amide linkages in proteins and polypeptides. Proteases are involved in almost every aspect of life. The functions of proteases[I.01] include hydrolysis of proteins and polypeptides for digestive and nutritional purposes, release of peptide hormones and neuromodulators from inactive precursors, activation of enzymes, for example clotting factors, and termination of biological responses by degradation of the message-transmitting peptide.

Four classes of proteases, each with a distinct catalytic mechanism, have been identified[I.02-I.03]. Classification as serine, aspartic, cysteine or metallo protease is based on the most significant catalytic functional group, or prosthetic group (in the case of metallo proteases) in the active site of the enzyme. TABLE I.01 lists examples of proteases in each mechanistic category.

SECTION I.2

SERINE PROTEASES AND THEIR MECHANISM OF PEPTIDE HYDROLYSIS

Serine proteases are involved in digestion, processing of peptide prohormones, thrombolysis and fibrinolysis, fertilization and blastocyst implantation[I.04-I.05]. Even though the physiological functions of serine proteases are diverse, all employ a common catalytic mechanism.

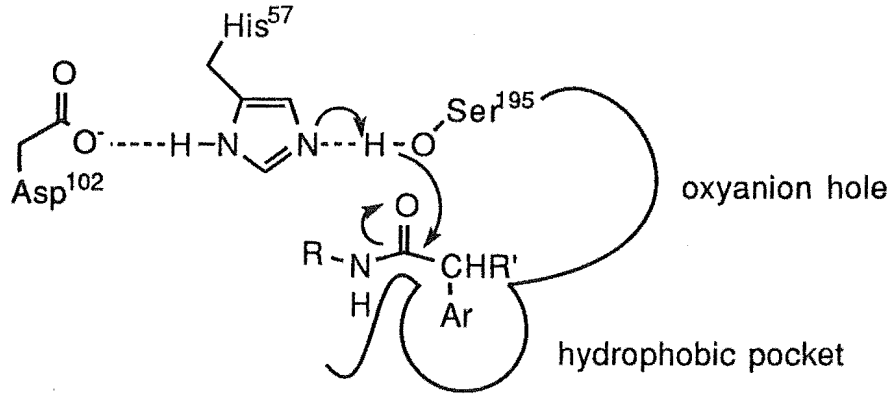
Chymotrypsin[I.06-I.07], a serine protease from the mammalian pancreas involved in digestion, is representative mechanistically of the whole class of serine proteases. Chymotrypsin has been studied intensively and more information is available on its mode of catalysis than for almost any other enzyme[I.08-I.09]. The active site has been well characterized by X-ray crystallography and intermediates of the hydrolysis reaction are known[I.06-I.09].

TABLE I.01: Examples of Proteases, Subdivided into Mechanistic Categories

Protease	Significant Active Site Groups	Representative Enzymes	Normal Function
Serine	Ser (hydroxyl) His (imidazole) Asp (carboxyl)	thrombin, plasma kallikrein, factors VIIa, IXa-XIIa, activated protein C	blood coagulation
		factors Clr, Cls D and B, C3 convertase	complement activation
		trypsin, chymotrypsin, pancreatic elastase	digestion
		entereokinase plasmin, plasminogen activator	fibrinolysis
		tissue kallikrein, post protein cleaving enzyme	hormone metabolism
		elastase, cathepsin G, most cell chymases, tryptases	phagocytosis
		ATP dependent proteases	protein turnover
Metallo	Zinc ion	angiotensin converting enzyme, aminopeptidases, renal dipeptidases	blood pressure regulation
		collagenase	tissue elasticity
		macrophage elastase	blood pressure regulation, peptide metabolism
		carboxypeptidase	digestion
Aspartic	Asp (carboxyl)	renin	blood pressure regulation
		HIV protease	HIV replication
		thermolysin, pepsin	digestion
Cysteine	Cys (thiol)	cathepsins B, H, L, calcium activated neutral proteases	protein turnover, bone resorption

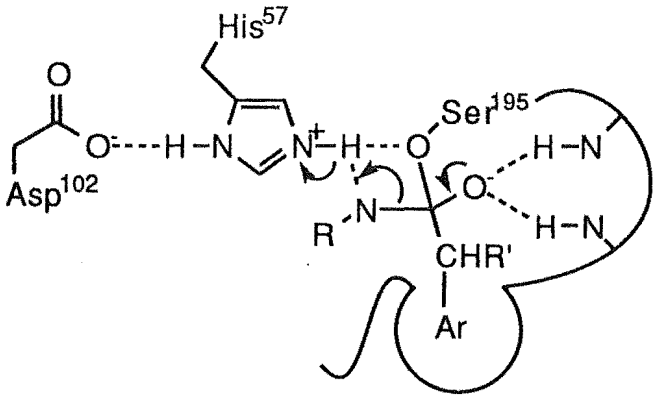
Chymotrypsin cleaves peptides on the carboxyl side of aromatic residues; phenylalanine, tyrosine and tryptophan. The aromatic side chain fits into a complementary hydrophobic pocket in the enzyme, known as the primary specificity pocket. The proposed mechanism of peptide hydrolysis by chymotrypsin is illustrated in SCHEME I.01. The driving force for hydrolysis is the catalytic triad of Asp^{102}, His^{57} and Ser^{195}. (The numbers 102, 57 and 195 denote the position of the amino acid residues Asp, His, and Ser, respectively in the polypeptide chain of the enzyme.)

SCHEME I.01: Proposed Mechanism of Peptide Hydrolysis by Chymotrypsin



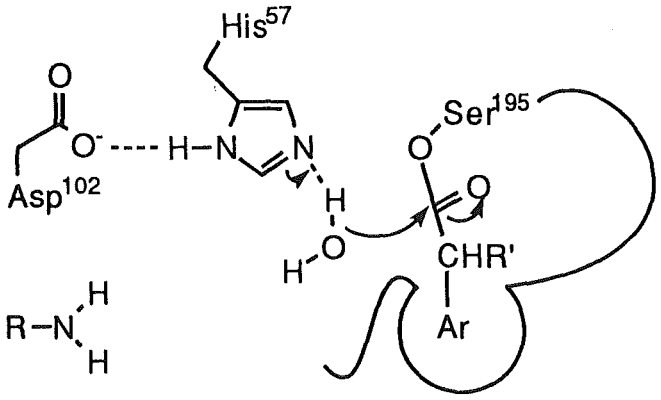
I.01 enzyme-substrate complex

step a



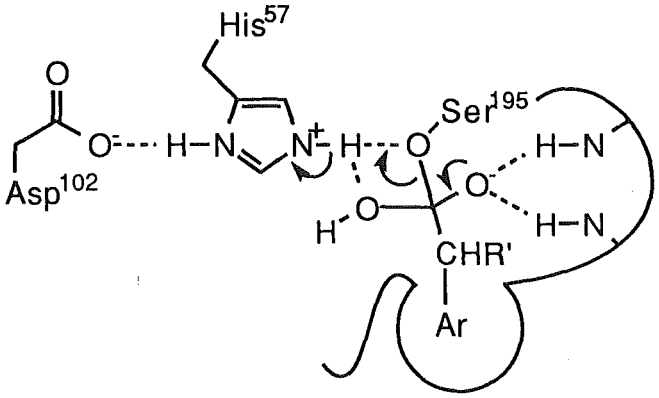
I.02 tetrahedral intermediate

SCHEME I.01 continued

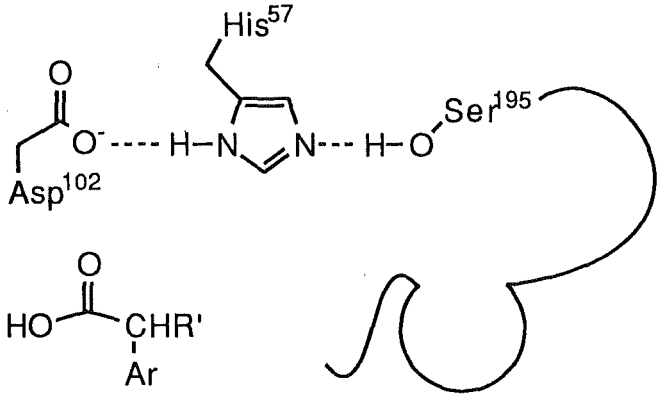


i.03 acyl enzyme

step b



i.04 tetrahedral intermediate



i.05 product and enzyme

Catalysis is initiated by nucleophilic attack of the Ser^{195} hydroxyl, which is activated by the imidazole of His^{57}, on the susceptible carbonyl carbon of the substrate (**i.01**, SCHEME I.01). This step forms the tetrahedral intermediate, the oxyanion of which is stabilized by hydrogen-bonding to the backbone NH groups of Gly^{193} and Ser^{195} (**i.02**, SCHEME I.01). Collapse of the tetrahedral adduct in the forward direction leads to release of the N-terminal amino acid and generation of an acyl enzyme (**i.03**, SCHEME I.01). Deacylation, facilitated by the above reactions operating in reverse (**i.04**, SCHEME I.01), yields the C-terminal amino acid and regenerates the enzyme (**i.05**, SCHEME I.01).

Chymotrypsin also displays esterase activity in addition to its peptidase (or amidase) activity.

SECTION I.3

INHIBITION OF PROTEASE ENZYMES

Proteases are strictly regulated by endogenous protease inhibitors[I.10]. However, a number of disease states, for example emphysema, inflammation, tumor metastasis, muscular dystrophy and hypertension, appear to be caused by excessive proteolytic activity brought on by abnormally low levels of endogenous protease inhibitor[I.04, I.11]. Serine proteases, in particular, have been implicated in emphysema, adult respiratory distress syndrome, rheumatoid arthritis, pancreatitis, inflammation and digestive disorders[I.04, I.12].

The involvement of serine proteases in a wide variety of physiological and pathological processes, coupled with their well studied mechanism of action, has made this class of enzyme attractive targets for the preparation of inhibitors.

Many strategies, including mechanism-based inactivation[I.13-I.19], alternate substrate inhibition[I.20-I.25], conformationally restricted peptides[I.26], transition state analogues[I.27] and affinity labels[I.28], have been used to develop effective and selective inhibitors of serine proteases.

Inhibitors acting in the enzyme active site are classically categorized as reversible or irreversible[I.13]. Reversible inhibitors closely resemble the normal substrate

and generally form a stable, non-covalent inhibitor-enzyme complex, whereas irreversible inhibitors form a covalent or particularly strong inhibitor-enzyme association. Inactivation refers to an irreversible inhibition process. Inactivation is viewed as a distinct advantage over reversible inhibition as a means of achieving a prolonged effect. Optimum activity of reversible inhibitors requires maintenance of a high inhibitor concentration at the active site. This can present dosage problems in clinical applications.

The main impetus for the development of enzyme inhibitors has been the rational design of drugs to specifically target key metabolic pathways under enzymatic control. However, inhibition studies have also revealed information regarding substrate specificity and the catalytic mechanism of enzymes.

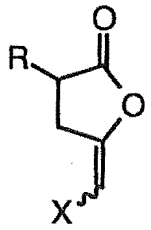
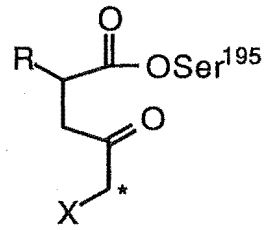
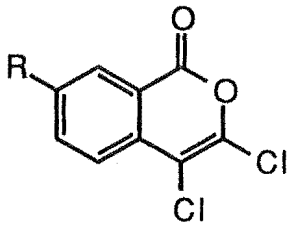
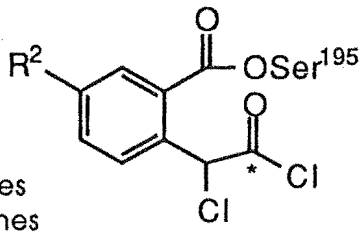
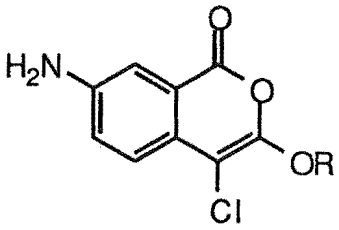
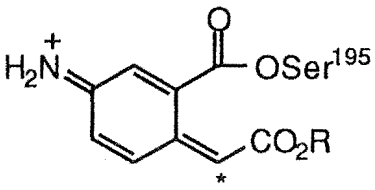
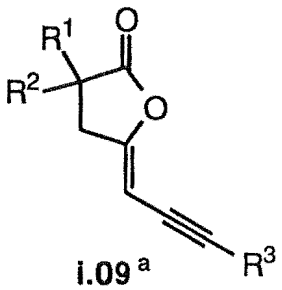
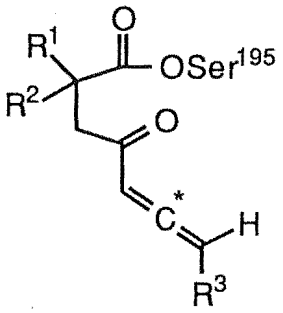
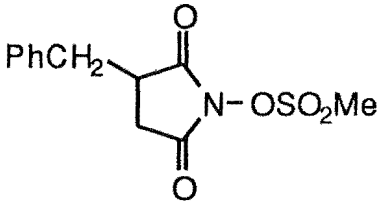
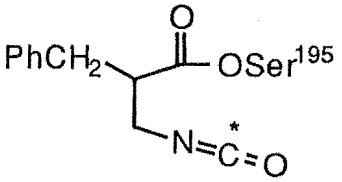
SECTION i.3.1

MECHANISM-BASED INACTIVATION

Mechanism-based inactivators[I.13] (also known as suicide inhibitors and k_{cat} inhibitors) are reasonably unreactive compounds which contain a latent reactive functionality. A mechanism-based inactivator is recognized by the target enzyme as a natural substrate. During catalysis, the latent reactive functionality in the inactivator is unmasked by the normal catalytic machinery of the target enzyme. The reactive group (generally an electrophilic site) becomes covalently bound to the enzyme (due to reaction with a nucleophilic residue in the enzyme). This renders the active site irreversibly blocked and the enzyme inactivated.

The design of mechanism-based inactivators for a target enzyme requires a knowledge of the enzyme mechanism and structure. As discussed earlier (*Section i.2*), the mechanism of serine protease catalyzed peptide hydrolysis has been extensively studied and consequently this class of enzyme is a popular target for mechanism-based inactivation[I.13-I.19]. TABLE I.02 shows enollactones[I.14] (**i.06**), chloroisocoumarins[I.15] (**i.07-i.08**), ynenol lactones[I.16] (**i.09**) and imides[I.17] (**i.10**), and their corresponding reactive groups, which are known mechanism-based inactivators of serine proteases.

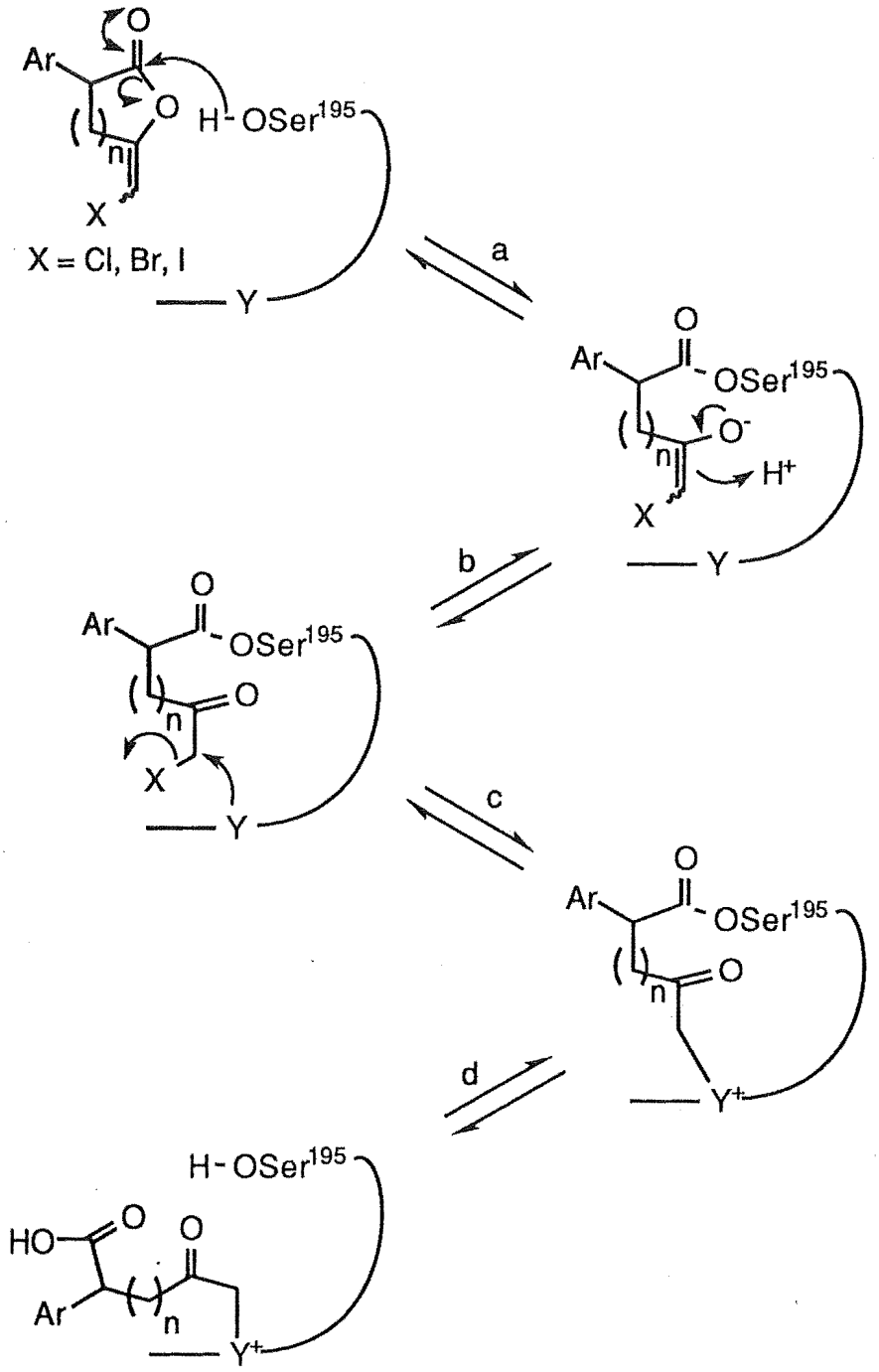
TABLE I.02: Examples of Mechanism-Based Inactivators of Serine Proteases

Masked Species	Target Enzyme(s)	Reactive Group
 **I.06** [a]	chymotrypsin	 **I.40** X = Cl, Br, I
 **I.07** [b]	elastases chymotrypsin trypsin coagulation enzymes complement enzymes	
 **I.08** [b]	human leukocyte elastase (HLE) chymases	
 **I.09** [a]	human leukocyte elastase (HLE)	
 **I.10**	human leukocyte elastase (HLE)	

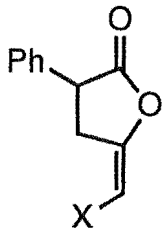
* Indicates position for covalent bond formation to the enzyme
a Nature of R determines efficiency of inactivation
b Nature of R determines enzyme specificity

The proposed mechanism of chymotrypsin inactivation by halo enollactones[1,14], a class of inhibitor studied in this thesis, is shown in SCHEME I.02. Acyl transfer to the Ser^{195} hydroxyl (step a) followed by a tautomeric shift (step b) reveals a reactive electrophilic α-halo ketone. Alkylation by an accessible active site nucleophilic amino acid residue, Y (probably His^{57}), renders chymotrypsin inactive (step c). The link to Ser^{195} may subsequently be hydrolyzed (step d).

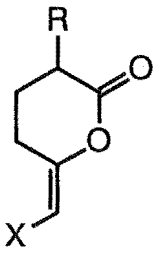
SCHEME I.02: Mechanism-Based Inactivation of Chymotrypsin by Halo Enollactones



A combination of computer graphics and molecular mechanics supports the proposed mechanism of chymotrypsin inactivation (SCHEME I.02) by the (R)- and (S)-α-phenyl- and α-benzyl-substituted bromo and iodo enollactones [I.29] (**i.11** and **i.12**).



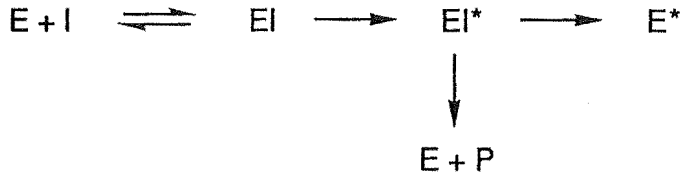
i.11 X = Br, I



i.12 X = Br, I
R = Ph, CH_2Ph

The process of enzyme (E) inactivation by a mechanism-based inactivator (I) can be represented by the simple steady state hypothesis shown in Equation I.01. The initially formed non-covalent enzyme-inactivator complex (EI) gives rise to the activated species (EI*) on specific enzyme action. A competition then exists between irreversible covalent inactivation (formation of E*) and the non-inactivation release of products (E + P).

Equation I.01



To establish the occurrence of mechanism-based inactivation a number of criteria must be satisfied[I.13]. The most important of these is that the reactive species, ultimately causing the irreversible enzyme inactivation, must be produced by the normal catalytic pathway of the enzyme while the inactivator is still bound at the active site.

There are many examples in nature of compounds that function as mechanism-based inactivators[I.30]. Some therapeutic drugs in use at present are mechanism-based

inactivators[I.13]. Many enzymes have been targeted for control by mechanism-based inactivators[I.13].

Apart from mechanism-based inactivators, the other main category of inactivators is affinity labels[I.13, I.28] (also known as active site directed irreversible inhibitors). Affinity labels are compounds that contain a reactive functional group, for example an α-halo ketone or isocyanate, and react directly with active site nucleophiles, generally via S_N2 alkylation or acylation. While useful *in vitro* for probing enzyme active sites, the major disadvantage of affinity labels is that they are capable of indiscriminate reactivity within the biological environment, resulting in toxicity and side effects. Many cancer chemotherapeutic agents are affinity labels.

In contrast, mechanism-based inactivators are particularly amenable to the design of highly specific, low toxicity drugs. The potential for generating the reactive species exclusively within the active site of the target enzyme imparts, in principle, a much higher degree of selectivity to mechanism-based inactivators than that exhibited by affinity labels. Another advantage of mechanism-based inactivation is that, in theory, only one inactivator molecule is needed per enzyme for inactivation. Non-specific interactions might occur however, if enzymes other than the target enzyme catalyze the production of a reactive species, or if a reactive species escapes from the enzyme before reacting.

SECTION I.3.2

ALTERNATE SUBSTRATE INHIBITION

Alternate substrate inhibitors[I.20-I.25] function as substrates of the target enzyme; however, instead of chemically reacting with the enzyme, the alternate substrate becomes bound so tightly to the active site that further access by natural substrate molecules is prevented.

Transition state analogues[I.13, I.27], another class of inhibitor, also bind tightly to the enzyme active site. Whereas alternate substrate inhibitors resemble the substrate and become covalently bound to the enzyme, transition state analogues are stable

compounds with a geometry or charge distribution resembling a transition state (or intermediate) of the reaction the target enzyme catalyzes. Transition state analogues form strong interactions with the active site which may, or may not, be covalent.

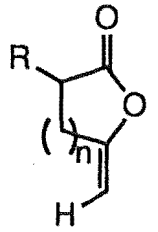
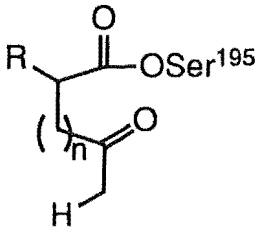
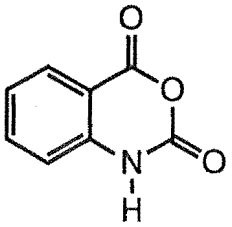
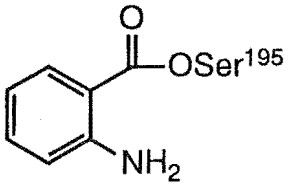
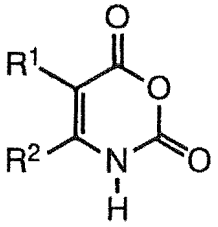
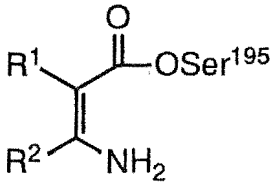
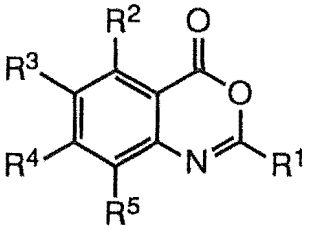
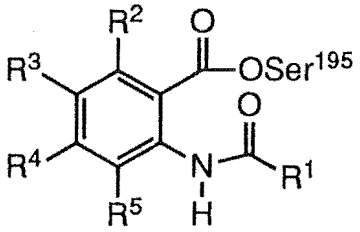
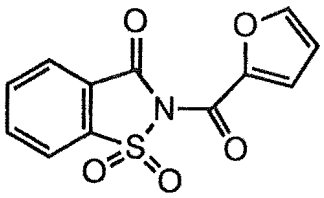
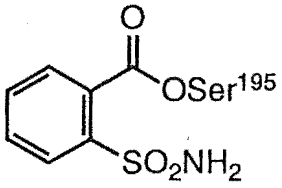
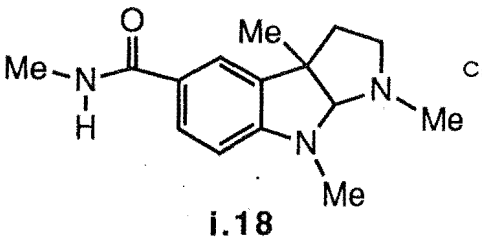
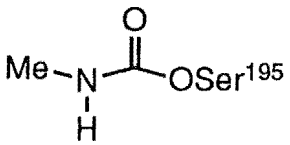
Alternate substrate inhibitors of serine proteases are simply substrates that form very stable acyl enzyme intermediates (*eg* **i.03**, SCHEME I.01, *Section I.2*), so that the enzyme remains inhibited for the life time of the acyl enzyme species. The acyl enzyme is a good target for rational drug design due to the broad range of compounds that can serve as substrates for serine proteases and form acyl enzyme intermediates, and also because a great deal is known from physical organic chemistry about ester reactivity and ways it can be controlled[I.30]. Disadvantages arise in therapeutic application as a consequence of the competitive and reversible nature of alternate substrate inhibition. For example, a high inhibitor concentration is required at the active site and potentially, repeated administrations are necessary.

Protio enollactones[I.20] (**i.13**), stabilized anhydrides[I.21] (**i.14** and **i.15**), benzoxazinones[I.22] (**i.16**) and *N*-acyl sacharrins[I.23] (**i.17**) which are alternate substrate inhibitors of serine proteases are shown in TABLE I.03. Physostigmine (**i.18**, TABLE I.03) is an alternate substrate inhibitor of cholinesterase found in nature[I.31].

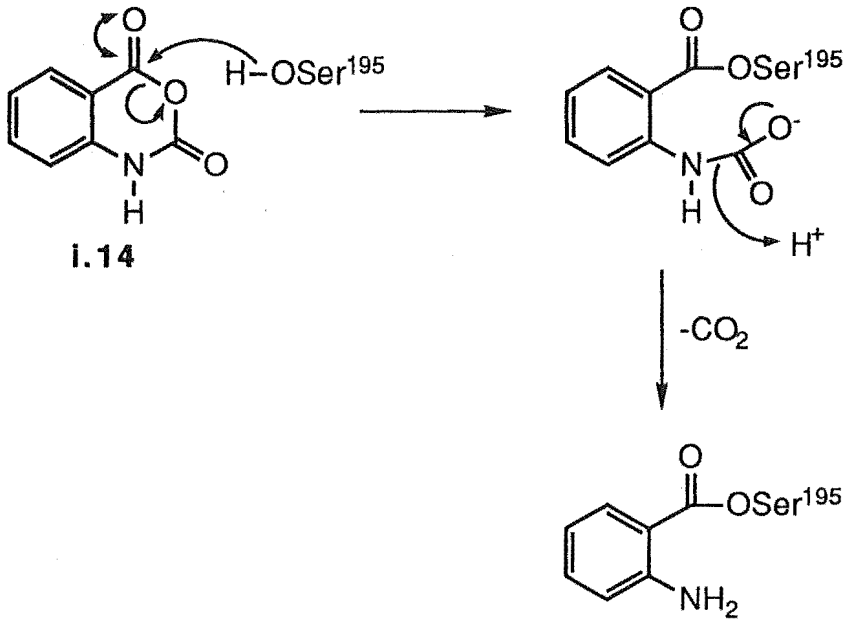
The protio enollactone alternate substrate inhibitors (**i.13**, TABLE I.03) of chymotrypsin are identical to the halo enollactone mechanism-based inactivators of chymotrypsin (**i.06**, TABLE I.02) except that they do not contain a latent reactive group.

The stability of the acyl enzymes is generally ascribed to an inherently low hydrolytic reactivity of the ester link or to an active site conformation that is unfavourable to catalyzed hydrolysis. For example, in the case of isatoic anhydride[I.21] (**i.14**, TABLE I.03 and SCHEME I.03) the slow hydrolysis of the acyl enzyme (**i.19**, TABLE I.03 and SCHEME I.03) is due to the electron releasing properties of the NH_2 group. An important feature of the proposed mechanism of inhibition (SCHEME I.03) is that initially the NH_2 group is masked (**i.14**) and it does not become expressed until the acyl enzyme (**i.19**) is formed.

TABLE I.03: Examples of Alternate Substrate Inhibitors of Serine Proteases

Alternate Substrate Inhibitor	Target Enzyme	Acyl Enzyme Species
 **i.13**	chymotrypsin	 **i.43**
 **i.14**	chymotrypsin	 **i.19**
 **i.15**	chymotrypsin pancreatic elastase	
 **i.16**	human leukocyte elastase (HLE)	
 **i.17**	chymotrypsin pancreatic elastase human leukocyte elastase (HLE)	
 **i.18**	cholinesterase	

SCHEME I.03: Alternate Substrate Inhibition of Chymotrypsin by Isatoic Anhydride



I.14 I.19

Alternate substrate inhibitors should interact with the enzyme in the manner of a normal substrate as shown in Equation I.02. The rate constant for deacylation, k_d, is significantly less for the alternate substrate than the natural substrate.

Equation I.02

$$E + S \underset{}{\overset{K_s}{\rightleftharpoons}} E.S \xrightarrow{k_a} E\sim S \xrightarrow{k_d} E + P$$

E	=	enzyme
S	=	alternate substrate
E.S	=	Michaelis complex
E-S	=	acyl enzyme intermediate
P	=	product

Physico chemical terms and enzyme specific interactions are important in determining inhibition[I.22]. Potency and stability are desirable for any potential therapeutic agent. Potency is achieved by rapid acylation combined with slow

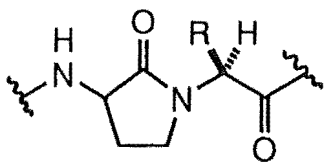
deacylation. Acyl enzyme stability is obtained by a combination of steric and electronic effects. Several alternate substrate inhibitors have been found to form acyl enzymes with life times over 10h[I.22]. The ability to control the duration of inactivation may be useful in the *in vivo* application of these inhibitors.

SECTION I.3.3

CONFORMATIONALLY RESTRICTED SYSTEMS

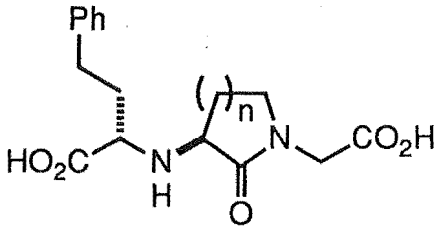
Generally, peptides exist in solution as an equilibrium mixture of conformers. Conformational restriction of peptides and amino acid analogues limits the number of conformations available[I.03, I.32-I.33]. Conformationally restricted peptide and amino acid analogues; for example, dipeptide lactams, *N*-methyl amino acids, α,α-disubstituted amino acids, β and γ bend mimics and others, have been used extensively to study the biologically important preferences of bioactive peptides[I.34]. Although not necessarily inhibitors in their own right, the incorporation of conformationally restricted peptides or amino acid analogues into known inhibitors can be advantageous. Potential benefits of conformational restriction in inhibitors include; increased metabolic stability due to decreased enzymatic degradation; increased potency due to stabilization of a bioactive conformer; and increased specificity due to elimination of conformers which inhibit other enzymes[I.03, I.35].

Considerable use has been made of lactams for achieving conformational constraint in bioactive peptides. For example, the incorporation of (**i.20**) into inhibitors of renin, an aspartic protease, has afforded compounds which are potent and resistant to degradation by chymotrypsin[I.36]. Inhibiton of renin is a therapeutic strategy for controlling hypertension[I.03, I.37].

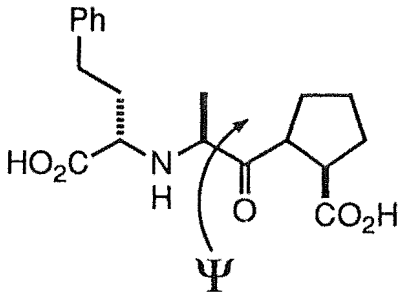


i.20

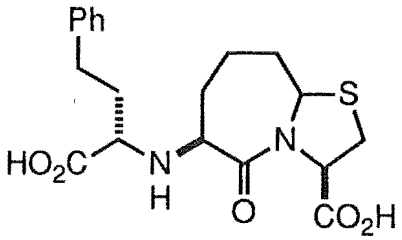
Inhibition of angiotensin converting enzyme (ACE), a metallo protease, is a viable route for treatment of hypertension and congestive heart failure. Studies of five-, six-, seven and eight-membered lactams (**i.21**), as conformationally restricted analogues of enalaprilate (**i.22**), an ACE inhibitor, allowed determination of Ψ (the torsion angle) in the bioactive conformation of enalaprilate[i.38]. This information was used to synthesize enalaprilate analogues (**i.23**) of increased potency, constrained to the optimum Ψ value[i.39].



i.21



i.22



i.23

Conformationally restricted analogues of chymotrypsin substrates have been used to explore the specificity and mechanism of substrate binding[i.08].

The alternate substrate inhibitors shown in TABLE i.03 (*Section i.3.2*)are essentially conformationally locked substrates of serine proteases. The mechanism-based inactivators shown in TABLE i.02 (*Section i.3.1*) represent conformationally locked substrates of serine proteases which contain a latent reactive group.

SECTION I.4

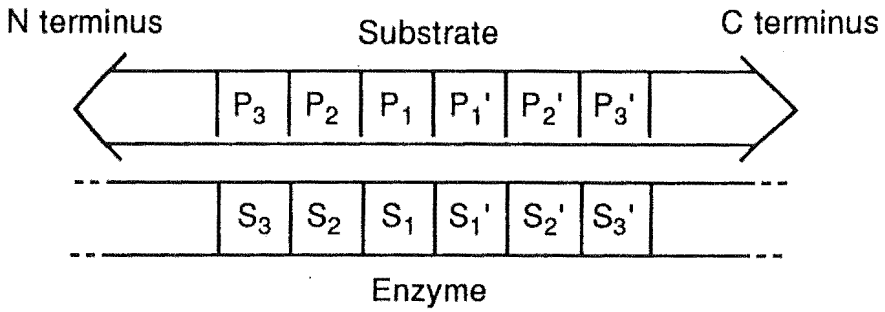
SPECIFICITY IN SERINE PROTEASE INHIBITORS

A major challenge in producing a therapeutically useful inhibitor is to obtain specificity. With respect to inactivation of serine proteases, this demands selective inactivation of one enzyme among a group of closely related enzymes. The three-dimensional structures of serine proteases are very similar and they all employ the same catalytic mechanism (*Section I.2*) in spite of a diversity of physiological functions.

Primarily, it is the primary specificity pocket, S_1, of the enzyme which determines the amino acid residues accepted by the active site and hence the point of cleavage. S_1 is a hydrophobic pocket in most serine proteases[I.07]. The size and shape of the pocket determine substrate specificity.

The binding site of a proteolytic enzyme for a polypeptide substrate is conveniently defined in terms of a series of subsites[I.40] (SCHEME I.04). Amino acid residues of the substrate P_n...P_3, P_2, P_1 and P_1', P_2', P_3'...P_n' are located in subsites S_n...S_3, S_2, S_1 and S_1', S_2', S_3'...S_n' of the enzyme, respectively. Cleavage occurs between P_1 and P_1'.

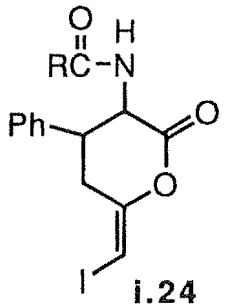
SCHEME I.04



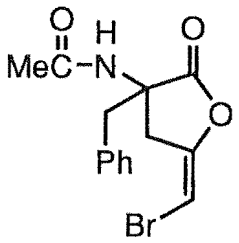
The S_1 specificity pocket in chymotrypsin can best accommodate a planar aromatic side chain, hence chymotrypsin cleaves peptide bonds on the carboxyl side of an aromatic amino acid residue. The S_1 specificity pocket in trypsin, which cleaves on the carboxyl side of arginine and lysine, is very similar to that in chymotrypsin except that Ser^{189} is replaced by the acidic amino acid Asp^{189}. Consequently, the basic side

chains of lysine and arginine at P_1 are stabilized when bound in the S_1 pocket. In elastase, which cleaves on the carboxyl side of glycine and alanine, the S_1 specificity pocket is partially occluded by the side chain of Val^{216} (Gly in trypsin and chymotrypsin), and the bottom is partly filled by the side chain of Thr^{226} (Gly in trypsin and chymotrypsin) leaving room for binding of Ala and Gly side chains at P_1, which are small.

In the design of specific inhibitors, the S_1 primary specificity pocket is very important. Specificity considerations were the motivation for the synthesis of the halo enollactones (**i.24** and **i.25**), which are mechanism-based inactivators of chymotrypsin[1.19].



i.24



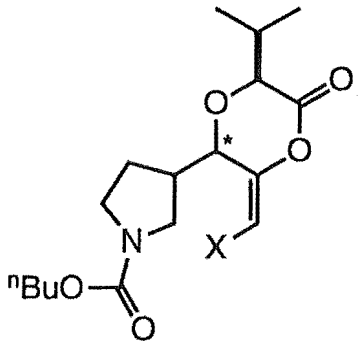
i.25

The halo enollactones (**i.24** and **i.25**) bear a close structural resemblance to phenylalanine derivatives which are often good substrates for α-chymotrypsin; however, they were prepared in racemic form only.

Although the S_1 specificity pocket is obviously important in substrate recognition and binding, it is not the only binding site. Secondary substrate binding sites may also exist and these provide the opportunity for increasing the binding specificity of a synthetic inactivator. For some aspartic proteases, the specificity is extended to seven (P_4-P_3') or more amino acids.

The specificity of affinity labels has been enhanced by attachment of the reactive group to an amino acid sequence recognized by the target enzyme[1.27].

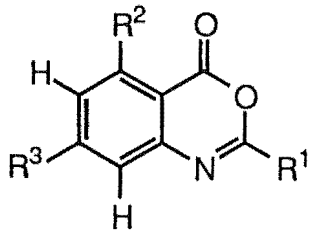
As a strategy for increasing specificity, halo enollactones and protio enollactones have been incorporated into pseudo dipeptides[1.24] (**i.26-i.29**). The pseudo dipeptide is based on the proline-valine sequences typically seen as the P_2-P_1 residues in elastase substrates.



	X	*
i.26	H	R
i.27	H	S
i.28	Br	R
i.29	Br	S

Whereas the protio enollactones (**i.26-i.27**) were not effective alternate substrate inhibitors, one of the bromo enollactone diastereoisomers (**i.29**) was a very effective inactivator of chymotrypsin and human leukocyte elastase (HLE), but not serine proteases of differing specificity. The observed specificity was superior to that found in other mono substituted halo enollactones.

Benefits have also been obtained on peptidyl substitution of 3,1-benzoxazin-4-ones, alternate substrate inhibitors of human leukocyte elastase (HLE)[1,22]. Dipeptide-substituted benzoxazinones (**i.31** and **i.32**) showed a 100-fold improvement in the rate constant for inhibition, K_I, compared with pyrrolidinyl-substituted benzoxazinone (**i.30**), and enhanced specificity is also expected.



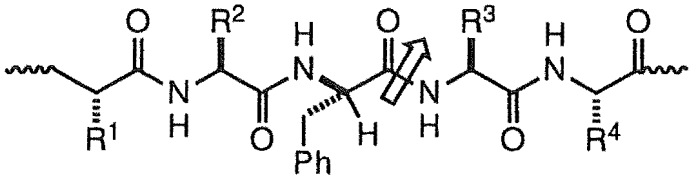
	R^1	R^2	R^3
i.30	1-pyrrolidinyl	H	H
i.31	Pro-Leu-NH_2	H	H
i.32	Pro-Leu-NH_2	Et	NH_2

It should be possible to target different serine proteases with a high degree of specificity by incorporation of the same inhibitor, for example halo enollactone or protio enollactone, into an oligopeptide specific for the target enzyme.

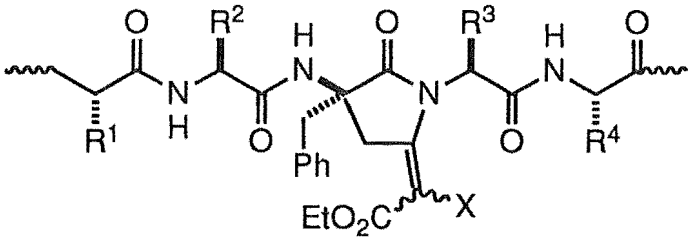
SECTION I.5

RESEARCH DESCRIBED IN THIS THESIS

This thesis is concerned with the development of a new class of potential specific alternate substrate inhibitors and mechanism-based inactivators of chymotrypsin. A normal chymotrypsin substrate is represented by (**I.33**) and the point of cleavage is indicated by the arrow. The proposed new class of mechanism-based inactivators and alternate substrate inhibitors of chymotrypsin is illustrated by the bromo enamino ester (**I.34**) and protio enamino ester (**I.35**), respectively.



I.33



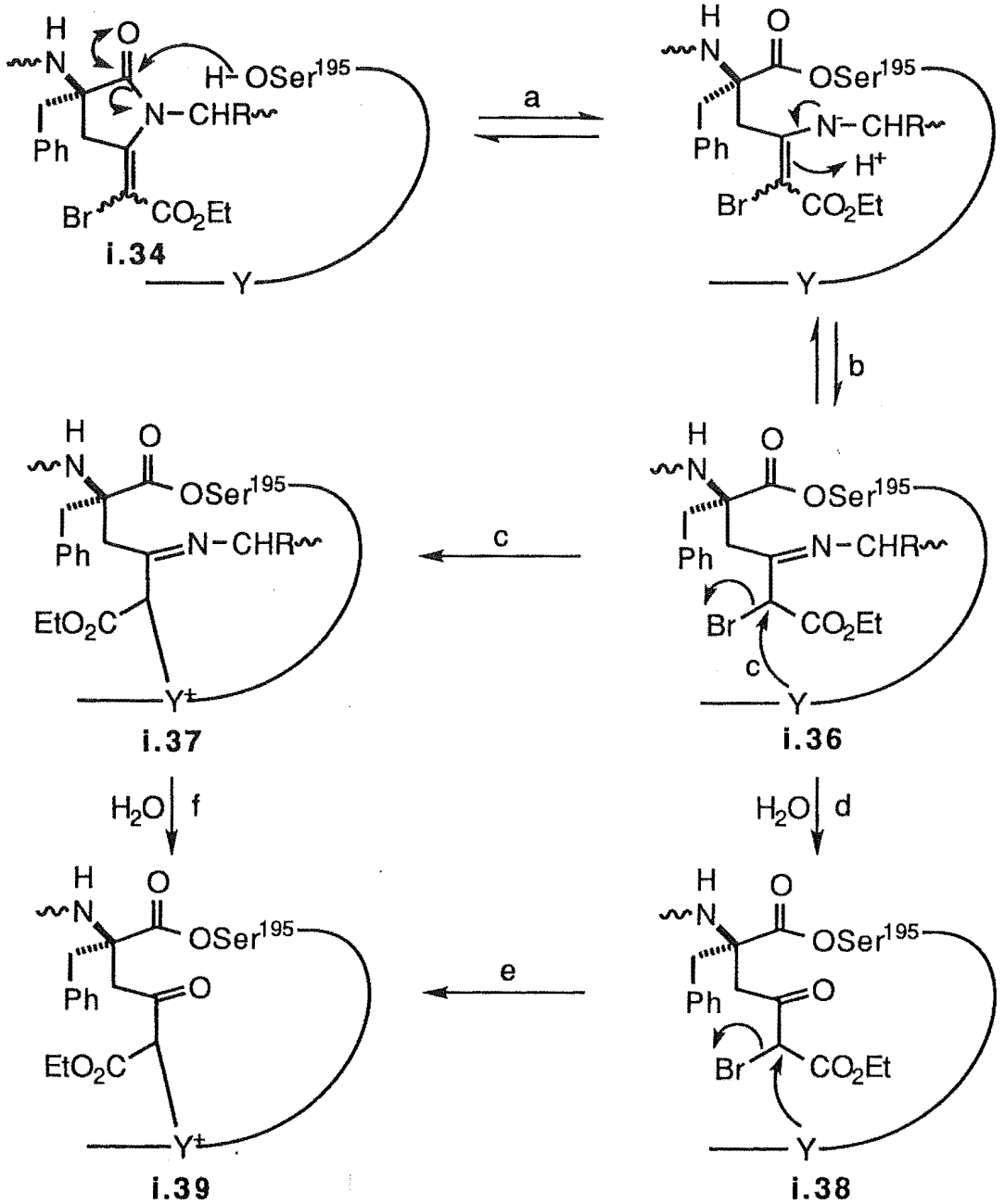
I.34 X = Br
I.35 X = H

The proposed inhibitors (**I.34** and **I.35**) have a benzyl group, analogous to the R group of phenylalanine, for recognition by chymotrypsin. Other amino acids could also be incorporated at this position. The enamino esters (**I.34** and **I.35**) form part of an oligopeptide with the same peptide backbone as a normal chymotrypsin substrate (**I.33**), hence there is the opportunity for increased binding specificity. The enamino esters (**I.34** and **I.35**) are also conformationally restricted systems.

The proposed mechanism of chymotrypsin inactivation by bromo enamino ester (**I.34**), (steps a, b, c, SCHEME I.05) parallels chymotrypsin inactivation by halo enollactones (steps a, b, c, SCHEME I.02, *Section I.3.1*) except that in this case the

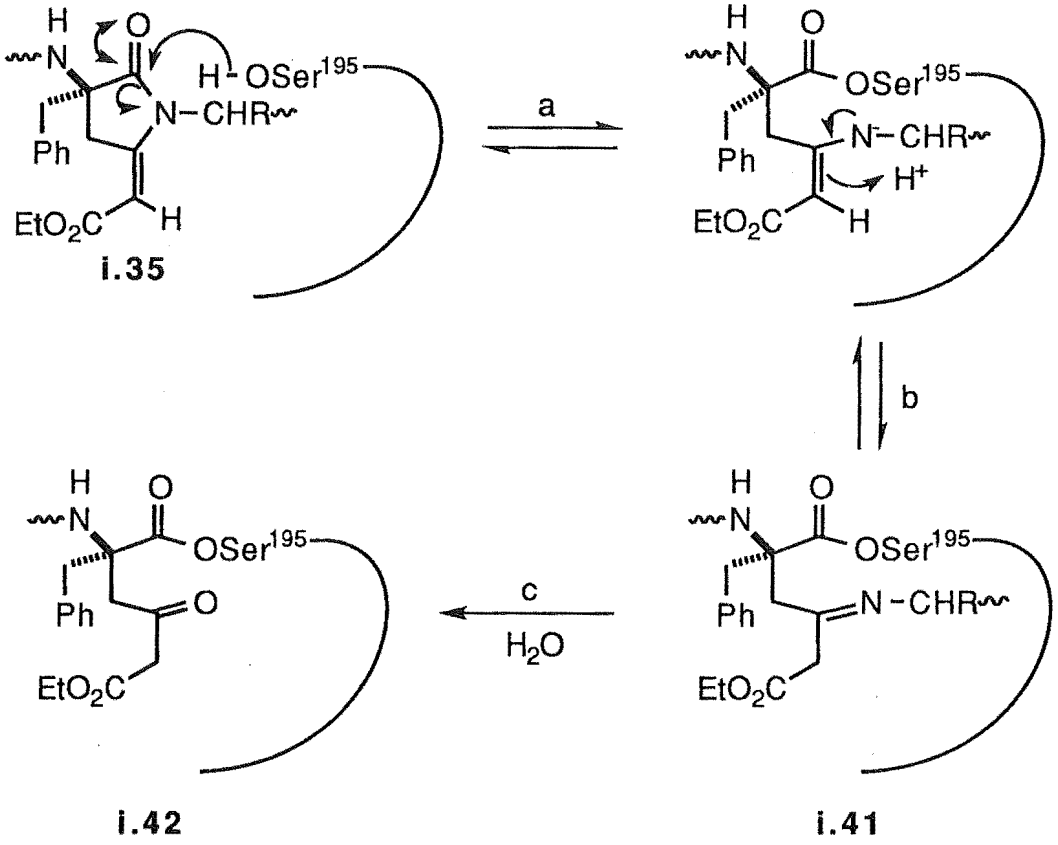
reactive species is an α-halo enamine (**i.36**, SCHEME I.05). Alternatively, hydrolysis of the α-halo enamine (**i.36**) leads to formation of the α-halo ketone (**i.38**) (step d, SCHEME I.05). This species is structurally very similar to known inactivators of chymotrypsin (**i.40**, TABLE I.02, *Section I.3.1*) and hence is expected to be alkylated by the nucleophilic active site residue, Y, to produce the inactivated enzyme (**i.39**, step e, SCHEME I.05). Alternatively, hydrolysis of (**i.37**) may occur to give (**i.39**) (step f, SCHEME I.05).

SCHEME I.05: Mechanism-Based Inactivation of Chymotrypsin by Enamino Ester (**i.34**)



The proposed mechanism of inhibition of chymotrypsin by protio enamino ester (**i.35**) is shown in SCHEME I.06. Formation of a stable acyl enzyme (**i.41**) (steps a and b, SCHEME I.06) will render (**i.35**) an effective alternate substrate inhibitor. Hydrolysis of (**i.41**) may occur leading to formation of (**i.42**) (step c, SCHEME I.06) which is structurally very similar to known stable acyl enzyme inhibitors of chymotrypsin (**i.43**, TABLE I.03, *Section I.3.2*).

SCHEME I.06: Alternate Substrate Inhibition of Chymotrypsin by Protio Enamino Ester (**i.35**)



A main goal in this work is the development of a general, versatile synthesis that can be used to introduce CH_2Ph and other amino acid R groups into the inhibitor, and different amino acids into the oligopeptide, in a stereo-controlled manner. Thus potentially, by judicious choice of substituents, different serine proteases could be targeted with a high degree of selectivity. The proposed inhibitors (**i.34** and **i.35**) have the same stereochemistry as natural chymotrypsin substrates (**i.33**).

The synthesis of the target bromo and protio enamino esters (**i.34** and **i.35**) uses two new procedures developed during the course of this work. The first of these is the bromo lactonization of a keto acid phosphorane, via a modified SCOOPY reaction, to form a halo enollactone, and the second is the insertion of an amino acid into an enollactone to form an enamino ester.

Chapter 1 deals with initial development work on bromo and chloro lactonization of keto acid phosphoranes. Chapters 2-4 deal with reactions of enollactones and β-keto esters, ultimately leading to the synthesis of target molecules of the type (**i.34** and **i.35**). Chapter 5 deals with trends observed in the mass spectra of keto ester and keto acid phosphoranes.

This thesis represents the first stage in the development of these new classes of serine protease inhibitors. Subsequent work will concentrate on replacement of the CO_2Et group with H or Me to give compounds which more closely resemble known inhibitors, and development of syntheses of six-membered and larger enamino ester inhibitors.

SECTION I.6

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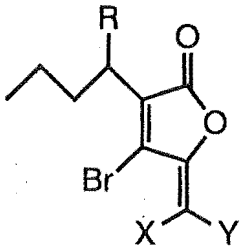
CHAPTER 1

SYNTHESIS OF HALO ENOLLACTONES

SECTION 1.1

INTRODUCTION

Halo enollactones are found in many natural products. For example, halo enollactones (**1.01**), which show antimicrobial and antifungal activity, have been isolated from the alga Delisea fimbriata[1.01].

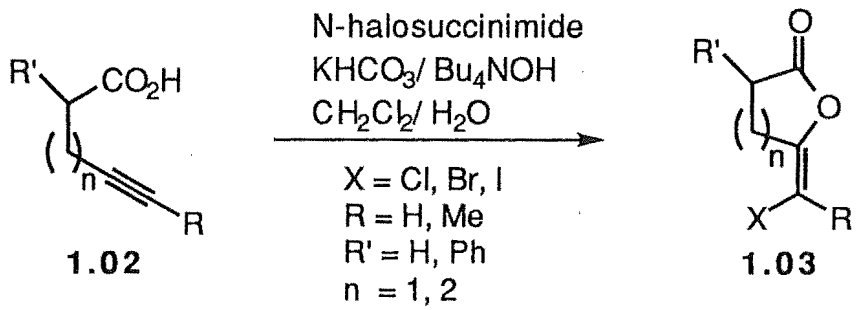


1.01

R = OAc		R = OH		R = H	
X	Y	X	Y	X	Y
Br	H	Br	H	Br	H
H	Br	H	Br	H	Br
I	H	I	H	Br	Br
H	I	H	I		
Cl	H	Cl	H		
H	Cl	H	Cl		
Br	Br	Br	Br		

The potential for halo enollactones to act as selective, efficient mechanism-based inactivators of serine proteases[1.13-1.14, 1.20] (discussed in detail in the Introduction, *Section 1.3.1*) has generated much interest in the properties of, and synthetic routes to, this class of compound. Halo enol-lactonization of acetylenic acids[1.02-1.03] (**1.02**) is the usual method of preparation of five- and six-membered halo enollactones (**1.03**) (SCHEME 1.01).

SCHEME 1.01



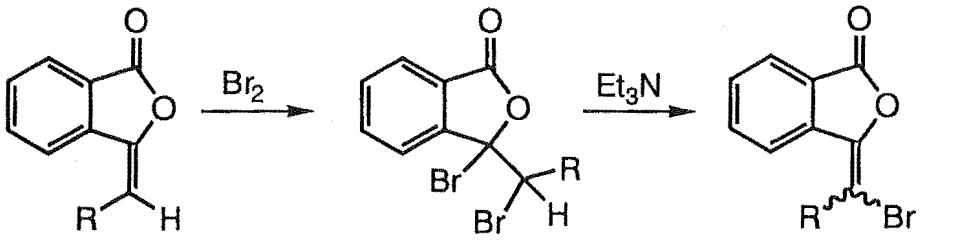
1.02 **1.03**

Six-membered enollactones are more effective inhibitors than five-membered enollactones[1.02-1.04]. Six-membered enollactones bind to chymotrypsin, a serine protease, with a 20- to 40-fold greater binding affinity, reflecting a better fit in the active site

relative to five-membered enollactones. Further, six-membered enollactones have a 100- to 200-fold higher activity as mechanism-based inactivators of chymotrypsin than five-membered enollactones. This result suggests that the reactive active site nucleophilic residue (step c, SCHEME I.02, in the Introduction) is more accessible to the electrophilic alkylating species (the α-halo ketone) derived from six-membered enollactones. However, five-membered enollactones are very useful probes for elucidating structure-activity relationships.

Other routes which involve halogenation and/or enol-lactonization of acetylenic acids have also been used to prepare five- and six-membered halo enollactones[1.02, 1.05]. Direct halogenation of protio enollactones (**1.04**) has met with limited success in the synthesis of halo enollactones (**1.05**)[1.02, 1.06] (SCHEME 1.02). This method represents one of the few reported syntheses of phthalic-based halo enollactones[1.06].

SCHEME 1.02



1.04 **1.05**

R = H or propyl

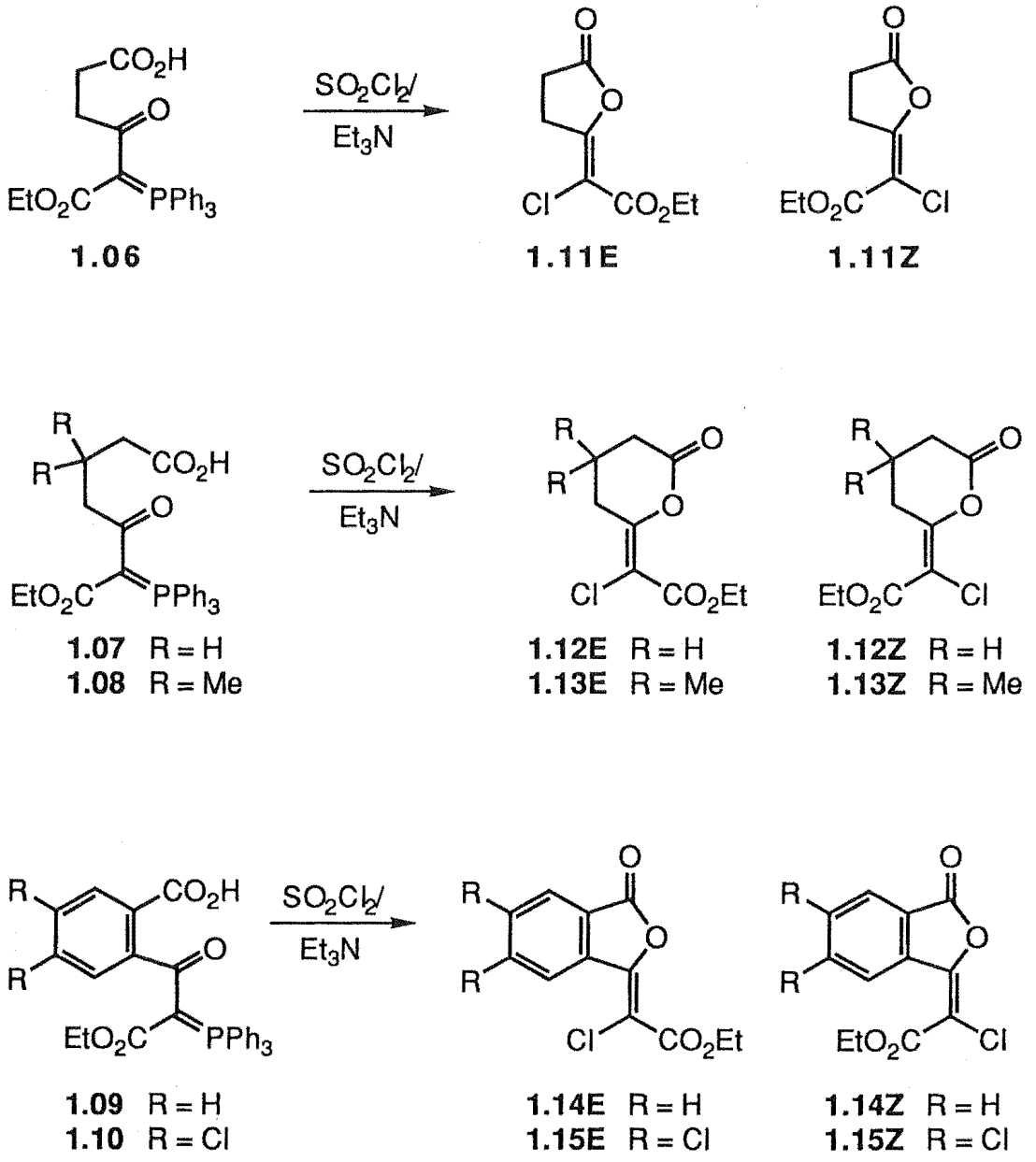
Phthalic halo enollactones (*eg* **1.05**, SCHEME 1.02) contain a latent reactive group and a hydrophobic aromatic group, comparable to the hydrophobic aromatic residue at the cleavage site in natural chymotrypsin substrates, and hence are expected to act as mechanism-based inactivators of chymotrypsin.

The above syntheses of halo enollactones tend to lack versatility and often give access to only one geometrical isomer. Another disadvantage is that the precursors are often time consuming and synthetically difficult to prepare.

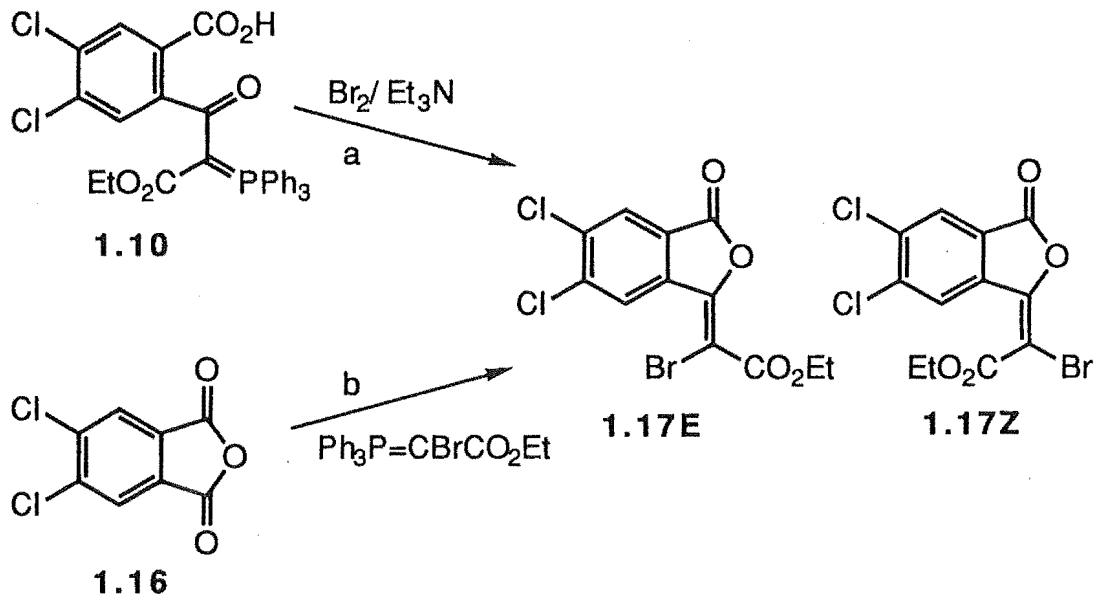
This chapter describes two new syntheses of halo enollactones (SCHEMES 1.03 and 1.04). Thus, E- and Z-chloro enollactones (**1.11-1.15**, SCHEME 1.03) and E- and Z-bromo

enollactones (**1.17**, pathway a, SCHEME 1.04) were prepared via halo enol-lactonization of keto acid phosphoranes (**1.06-1.10**), and E- and Z-bromo enollactones (**1.17**) were also prepared via reaction of 4,5-dichlorophthalic anhydride (**1.16**) with $Ph_3P{=}CBrCO_2Et$ (pathway b, SCHEME 1.04).

SCHEME 1.03



SCHEME 1.04

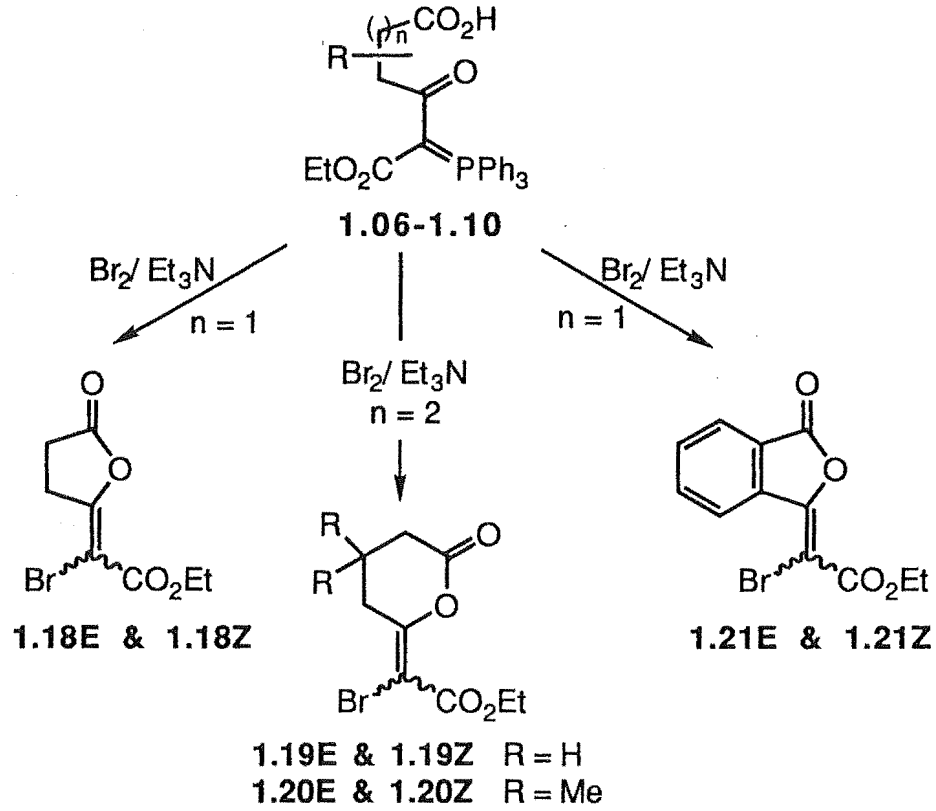


The halo enol-lactonization and anhydride/ylide reactions reactions will allow the preparation of new potential mechanism-based inactivators of chymotrypsin and other serine proteases. With respect to the target molecule (**i.34**, discussed in *Section I.5* in the Introduction), the reactions represent a means of incorporating latent reactivity.

By contrast to traditional methods for synthesis of halo enollactones (SCHEMES 1.01 and 1.02), the new reactions (SCHEMES 1.03 and 1.04) proceed in good yields. The halo enol-lactonization and anhydride/ylide reactions have previously been used[1.07-1.08] for the synthesis of E- and Z-bromo enollactones (**1.18-1.21**, SCHEME 1.05) and E- and Z-phthalic-based bromo enollactone (**1.21**), respectively.

The halo enol-lactonization (SCHEME 1.03 and pathway a, SCHEME 1.04) and anhydride/ylide (pathway b, SCHEME 1.04) reactions are related to the SCOOPY reaction (*ie*; α-Substitution plus Carbonyl Olefination via β-Oxido Phosphorous Ylides), which is also known as the Schlosser modification[1.09-1.11], and the Wittig anhydride carbonyl olefination reaction[1.12]. The SCOOPY reaction and the Wittig anhydride carbonyl olefination reaction are extensions of the Wittig reaction.

SCHEME 1.05

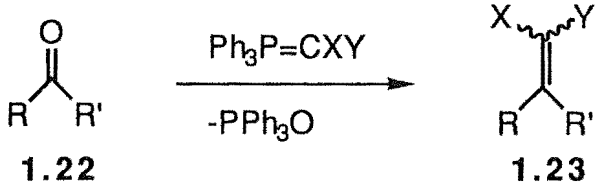


SECTION 1.2

WITTIG REACTION

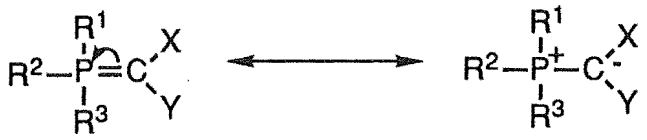
The Wittig reaction[1,13], since its discovery in 1953, has become one of the most widely used reactions in organic synthesis. The classic Wittig reaction is the reaction of a phosphorous ylide with the carbonyl of an aldehyde or ketone (**1.22**) to form a carbon-carbon double bond (**1.23**) (SCHEME 1.06).

SCHEME 1.06



Phosphorous ylides[1,14], hereafter referred to as ylides, are molecules in which a carbanion is directly attached to a phosphorous atom bearing a high degree of positive charge (SCHEME 1.07).

SCHEME 1.07



Ylides are classified as stabilized, semi-stabilized or non-stabilized depending on the substituent at the nucleophilic carbon (C-1). Stabilized ylides have strongly conjugating substituents; X (and/or Y) is CO_2R, CN, SO_2Ph and tend to form E-alkenes on reaction with aldehydes and ketones. Semi-stabilized ylides have mildly conjugating substituents; X (and/or Y) is phenyl, allyl. Non-stabilized ylides lack conjugating substituents and generally give rise to Z-alkenes. Ylide reactivity is also dependent on the substituents on phosphorous; usually $R^1 = R^2 = R^3 = Ph$.

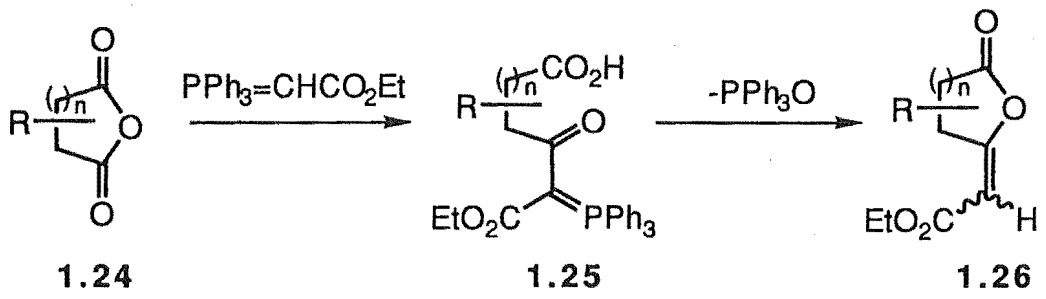
The Wittig reaction uses mild reaction conditions compatible with labile functional groups and allows control of stereochemistry about the double bond. However, the reaction mechanism is still under investigation and is far from fully understood[1,13].

SECTION 1.3

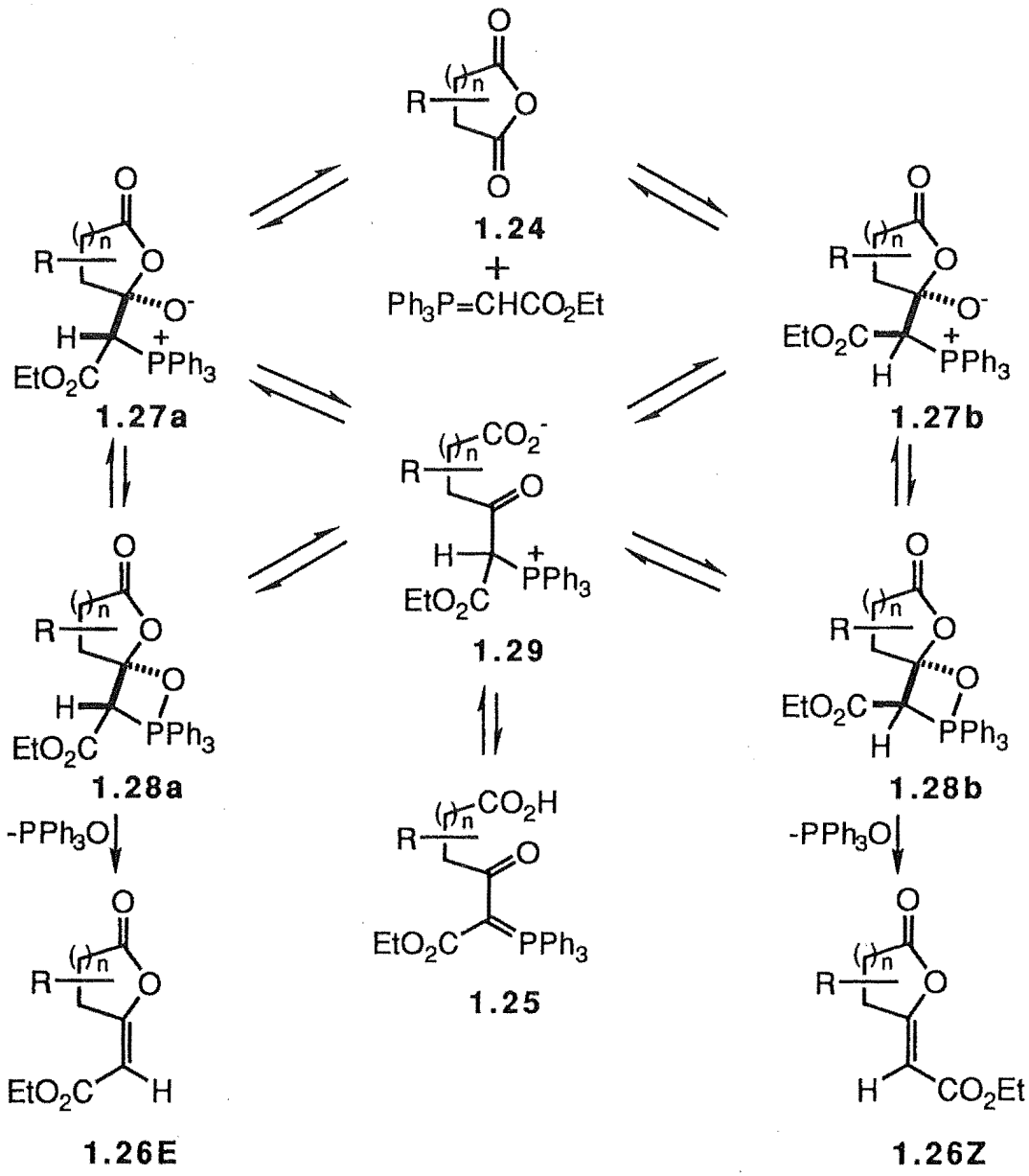
WITTIG ANHYDRIDE CARBONYL OLEFINATION REACTION

Synthesis of enollactones (**1.26**) via reaction of an anhydride (**1.24**) and stabilized ylide (SCHEME 1.08), the so-called Wittig anhydride carbonyl olefination reaction[1,12], represents an important extension of the Wittig reaction. The reaction is a general, high yielding procedure for the synthesis of five-, six- and seven-membered enollactones as well as phthalic-based enollactones. The proposed reaction mechanism is shown in SCHEME 1.09. Initial attack on the anhydride by the ylide gives one of two possible betaine intermediates (**1.27a** and **1.27b**). The betaines (**1.27a** and **1.27b**) may then form

SCHEME 1.08



SCHEME 1.09: Proposed Mechanism of the Wittig Anhydride Carbonyl Olefination Reaction



oxaphosphetanes (**1.28a** and **1.28b**, respectively). Loss of triphenylphosphine oxide from oxaphosphetane (**1.28a**) yields E-enollactone (**1.26E**), while loss of triphenylphosphine oxide from oxaphosphetane (**1.28b**) yields Z-enollactone (**1.26Z**). Alternatively, the betaine intermediates (**1.27a** and **1.27b**) may ring open to form an unstable phosphonium salt[1.15] (**1.29**) which rapidly isomerizes to the more stable, isolable keto acid phosphorane (**1.25**). Keto acid phosphoranes are synthetic intermediates to enollactones[1.16], allenes[1.17] and acetylenes[1.18], and are also of interest with respect to the extent of π-localization and modes of hydrogen-bonding[1.19].

The factors determining the product ratio of E- and Z-enollactones in the Wittig anhydride carbonyl olefination reaction are unclear. The Wittig anhydride carbonyl olefination reaction of succinic anhydride with $Ph_3P{=}CHCO_2Et$ gives rise exclusively to the E-enollactone.

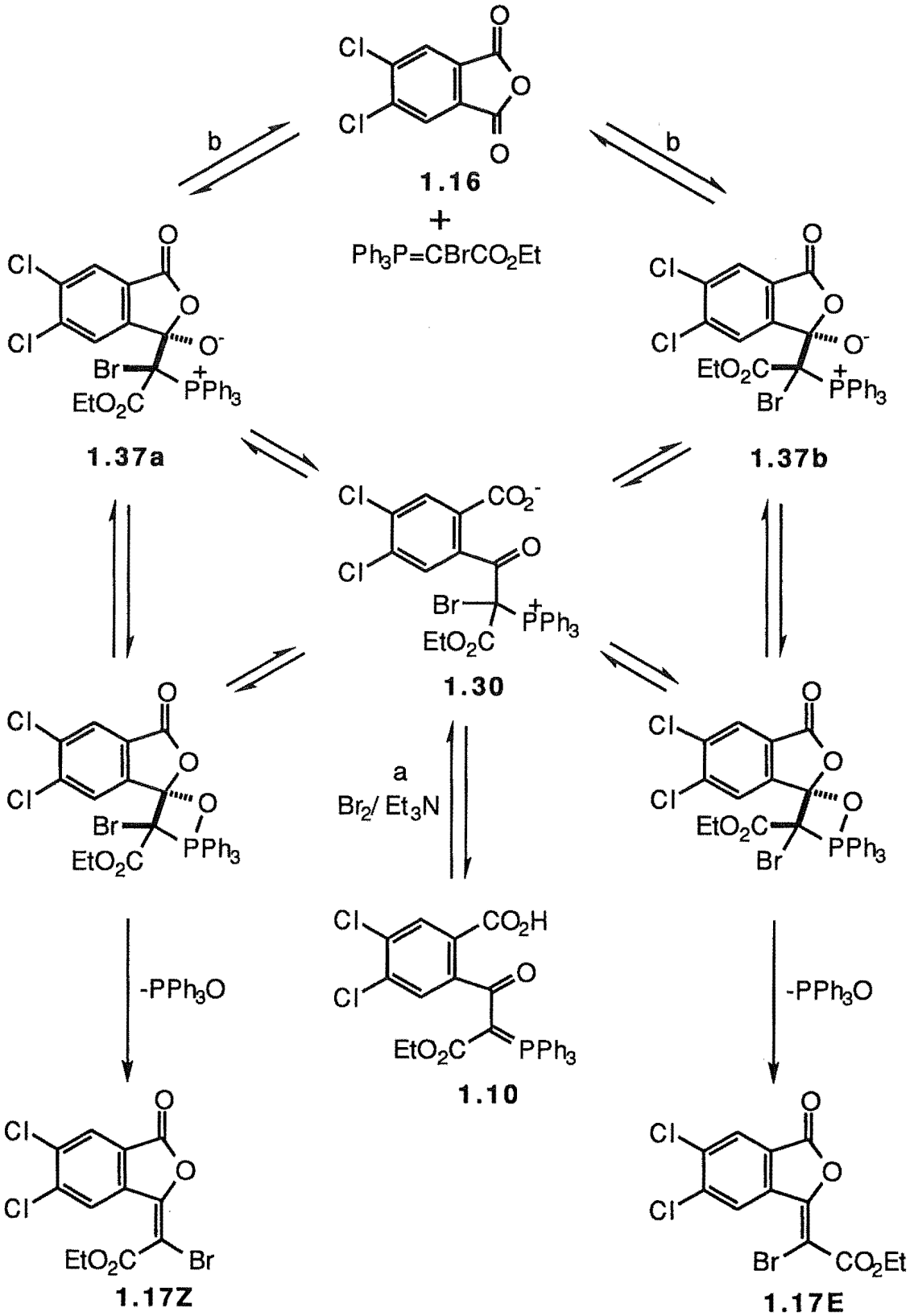
SECTION 1.4

SYNTHESIS OF BROMO ENOLLACTONES VIA REACTION OF ANHYDRIDE WITH $Ph_3P{=}CBrCO_2Et$

The Wittig anhydride carbonyl olefination reaction shown in SCHEMES 1.08 and 1.09 was adapted to allow the synthesis of E- and Z-dichlorophthalic bromo enollactones (**1.17E** and **1.17Z**, respectively) by use of the bromo ylide; $Ph_3P{=}CBrCO_2Et$ (pathway b, SCHEME 1.04). Thus, dichlorophthalic anhydride (**1.16**) and $Ph_3P{=}CBrCO_2Et$ (1.1 equivalent), dissolved in CH_2Cl_2, were stirred at 20 °C for 5h to yield the crude E- and Z-dichlorophthalic bromo enollactones (**1.17E** and **1.17Z**, respectively), in a ratio of 1 E : 4 Z, by ^1H NMR spectroscopy (E/Z assignments are discussed later in *Section 1.8*). The isomers were separated by radial chromatography on silica to give a combined yield of 61%. The proposed reaction mechanism (pathway b, SCHEME 1.10) is analogous to the mechanism of the Wittig anhydride carbonyl olefination reaction (SCHEME 1.09).

The formation of halo enollactone from reaction of $Ph_3P{=}CBrCO_2Et$ with an anhydride proceeds for phthalic- and 4, 5-dichlorophthalic-anhydride, but not for the less reactive succinic- and glutaric-anhydrides.

SCHEME 1.10: Proposed Mechanism for the Preparation of Bromo Enollactone (**1.17**) via Bromo Enol-Lactonization of the Keto Acid Phosphorane (Pathway a) and from the Anhydride/$Ph_3P{=}CBrCO_2Et$ Reaction (Pathway b):



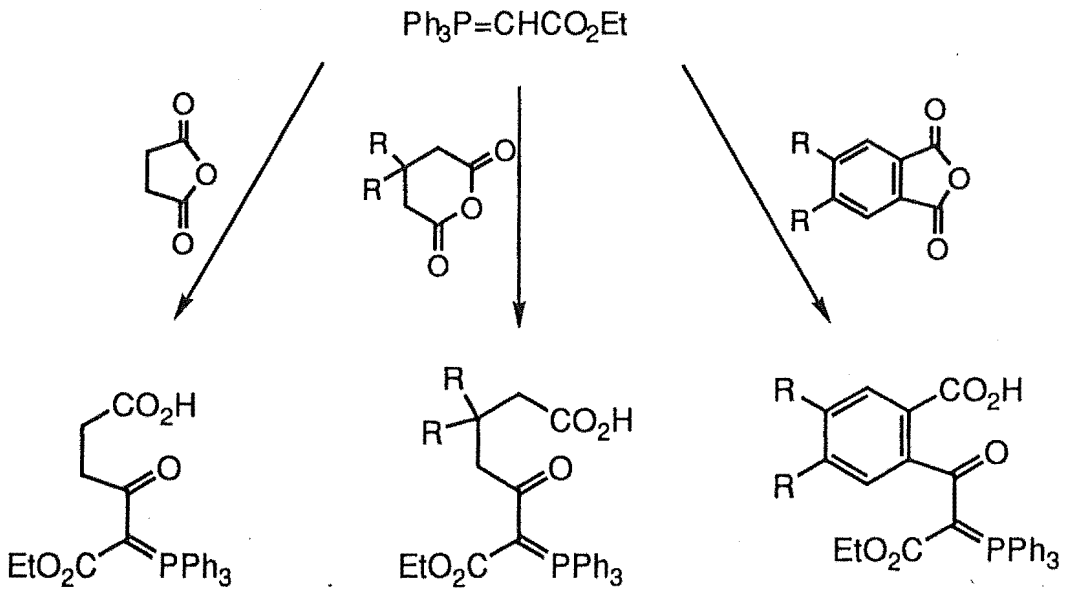
Thus, although representing a convenient procedure for the synthesis of E- and Z-phthalic-based bromo enollactones (**1.17** and **1.21**), the reaction does not appear to be generally applicable to the synthesis of succinic- and glutaric-based halo enollactones. Halo ylides are less reactive than $Ph_3P{=}CHCO_2Et$ due to decreased nucleophilicity at C-1, the site of reaction.

SECTION 1.5

SYNTHESIS OF HALO ENOLLACTONES VIA HALO ENOL-LACTONIZATION OF KETO ACID PHOSPHORANES

E- And Z-halo enollactones (**1.11-1.15, 1.17**) were also prepared via the halo enol-lactonization of keto acid phosphoranes (**1.06-1.10**) (SCHEME 1.03 and pathway b, SCHEME 1.04). This method avoids the initial and presumably slow step of ylide attack on the anhydride required for halo enollactone formation in the anhydride/$Ph_3P{=}CBrCO_2Et$ reaction (pathway b, SCHEME 1.10). The reactions required the independent synthesis of the key keto acid phosphoranes (**1.06-1.10**) via reaction of the appropriate cyclic anhydride with $Ph_3P{=}CHCO_2Et$ (SCHEME 1.11)[1.16].

SCHEME 1.11: Preparation of Keto Acid Phosphoranes



1.06

1.07 R = H
1.08 R = Me

1.09 R = H
1.10 R = Cl

The stable keto acid phosphoranes (**1.06-1.08**) were recrystallized, dissolved in CH_2Cl_2 and treated with SO_2Cl_2 (1.5 equivalent) and triethylamine (1.5 equivalent), at -78 °C. After 30min at -78 °C, the solutions were allowed to warm to 20 °C. Radial chromatography yielded pure E- and Z-chloro enollactones (**1.11**, **1.12** and **1.13**) in high yield (92%, 73% and 70%, respectively). The E and Z isomers were not separated by radial chromatography in this instance (E/Z assignments are discussed later in *Section 1.8*).

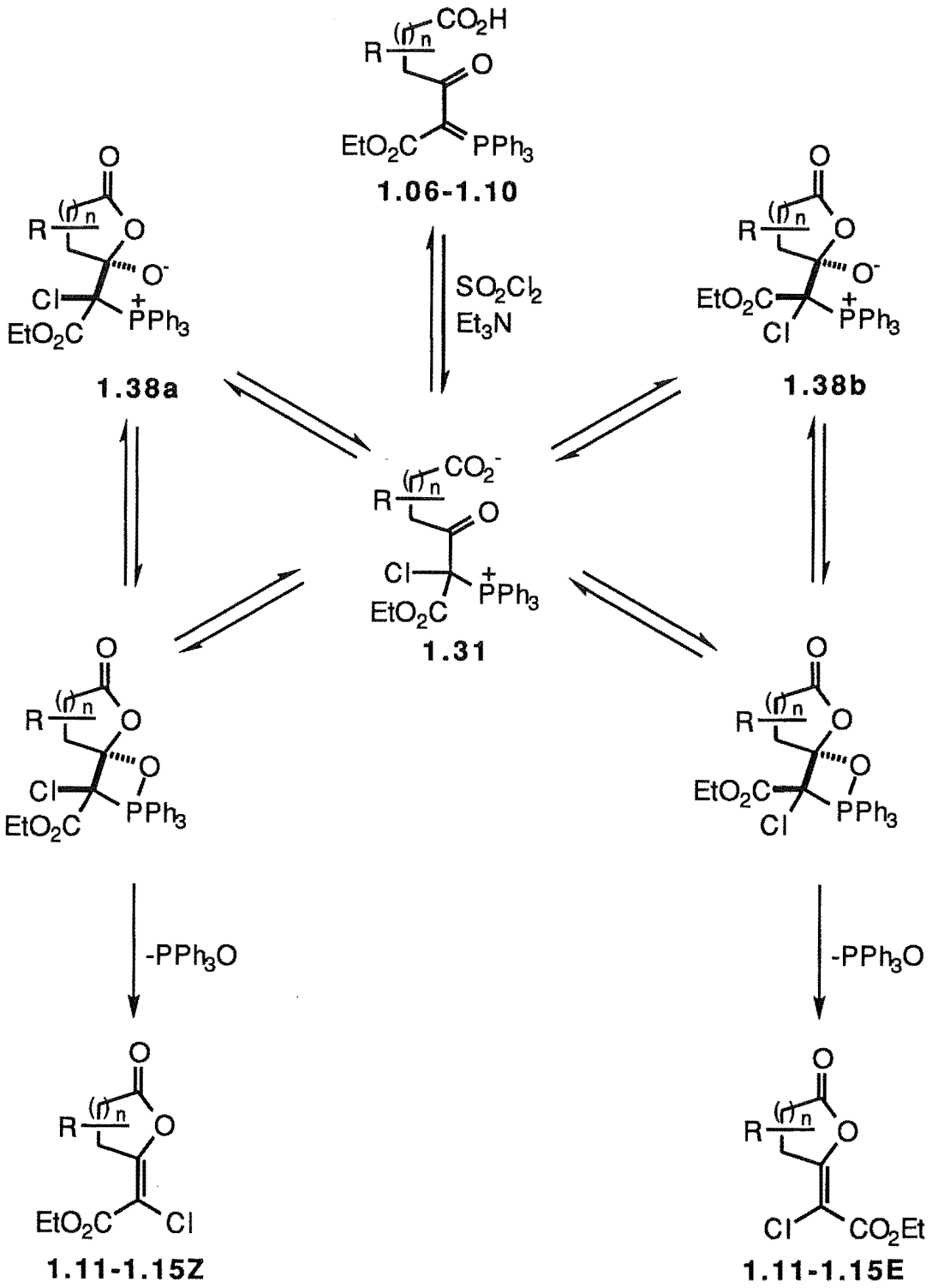
Phthalic-based keto acid phosphoranes (**1.09-1.10**), although observable by ^1H NMR spectroscopy at low temperatures, proved too reactive to isolate. Therefore, solutions of anhydride and $Ph_3P{=}CHCO_2Et$ in CH_2Cl_2 were stirred at 0 °C for 15min to allow formation of the keto acid phosphoranes (**1.09-1.10**). At this time, SO_2Cl_2 (1.5 equivalent) and triethylamine (1.5 equivalent) were added and the solution was stirred at 0 °C for 1h. Purification by radial chromatography yielded E- and Z-chloro enollactones (**1.14** and **1.15**) in high yield (62% and 93%, respectively). The E and Z isomers of the chloro enollactones (**1.14** and **1.15**) were separated by radial chromatography. However, for Z-phthalic chloro enollactone (**1.14Z**) a second chromatographic step was necessary to remove unreacted phthalic anhydride (E/Z assignments are discussed later in *Section 1.8*).

For the synthesis of bromo enollactone (**1.17**) via bromo enol-lactonization of keto acid phosphorane (**1.10**), a solution of anhydride and $Ph_3P{=}CHCO_2Et$ in CH_2Cl_2 was stirred at 20 °C for 10min at which time Br_2 (0.7 equivalent) and triethylamine were added. After 30min at

20 °C, the reaction mixture was purified by radial chromatography to yield E- and Z-bromo enollactones (**1.17E** and **1.17Z**, respectively) in a combined yield of 44%. The ratio of E- (**1.17E**) and Z- (**1.17Z**) bromo enollactones was the same as that obtained by the direct reaction of dichlorophthalic anhydride with $Ph_3P{=}CBrCO_2Et$ (pathway b, SCHEME 1.10); *ie*, 1 E : 4 Z. This indicates that a common reaction mechanism is likely for the two reactions, with a different method of producing the key halo phosphonium salt intermediate (**1.30**, SCHEME 1.10). The proposed reaction mechanism for the bromo enol-lactonization reaction and the anhydride/ylide reaction is shown in SCHEME 1.10. The two methods for the synthesis of phthalic bromo enollactone (**1.21**, SCHEME 1.05) also yielded the same E/Z isomer ratio. The mechanism proposed for the chloro enol-lactonization of keto acid

phosphoranes (**1.06-1.10**) (SCHEME 1.12) is analogous to the mechanism for bromo enol-lactonization (SCHEME 1.10).

SCHEME 1.12: Proposed Mechanism for the Synthesis of E- and Z-Chloro Enollactones (**1.11-1.15**) via Chloro Enol-Lactonization of Keto Acid Phosphoranes (**1.06-1.10**)

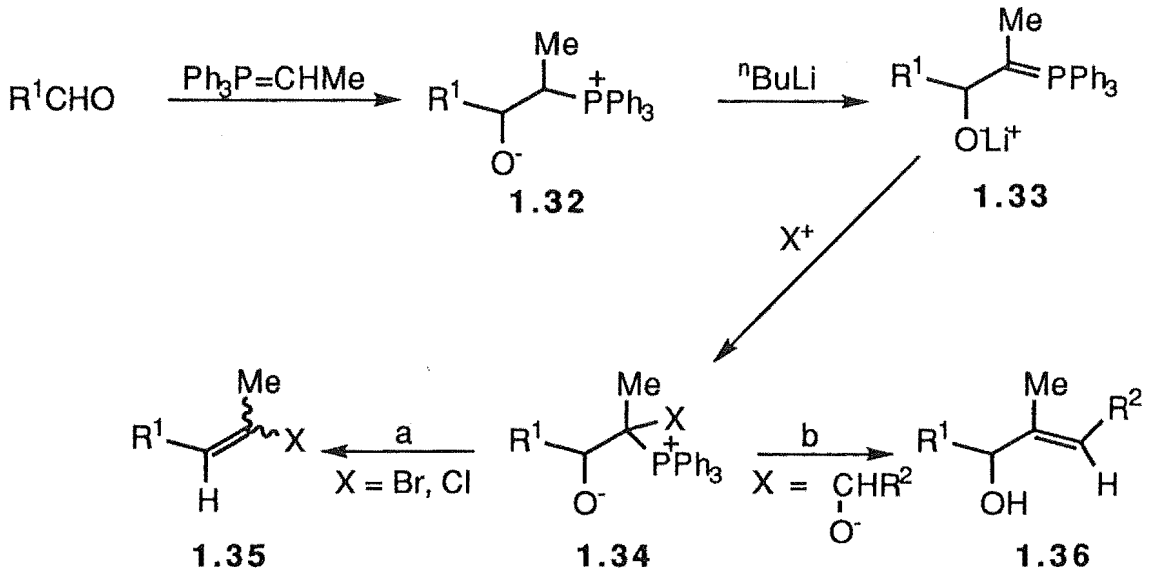


SECTION 1.6

SCOOPY REACTION

The bromo and chloro enol-lactonization reactions (pathway b, SCHEME 1.10 and SCHEME 1.12, respectively) represent extensions of the SCOOPY reaction[1.09-1.11] (pathway a, SCHEME 1.13). In the SCOOPY reaction an initially formed betaine (**1.32**), derived from an aldehyde and a non-stabilized ylide, is treated with ^{n}BuLi at low temperature to give a β-oxido ylide (**1.33**). Reaction with a second aldehyde (X^{+} = $R^{2}CHO$) then gives the allylic alcohol (**1.36**) stereoselectively, via the betaine (**1.34**) (pathway b, SCHEME 1.13). Halogen electrophiles[1.09-1.10], for example N-chlorosuccinimide, Br_2 or $FClO_3$, yield the analogous vinyl halides (**1.35**) (pathway a, SCHEME 1.13). The β-oxido ylide route to olefins (**1.36**) allows the joining of three components in one operation, such that the oxygen of the first aldehyde is retained, whereas that of the second aldehyde is eliminated as triphenylphosphine oxide[1.09].

SCHEME 1.13: SCOOPY Reaction



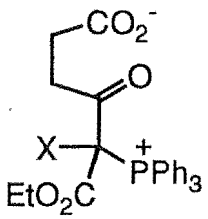
With the chloro and bromo enol-lactonization reactions (SCHEME 1.12 and pathway b, SCHEME 1.10, respectively) the β-oxido ylide (**1.33**) is by-passed and a betaine (**1.31**,

SCHEME 1.12 and **1.30**, SCHEME 1.10) analogous to (**1.34**) (SCHEME 1.13) is produced on reaction of a keto acid phosphorane (**1.06-1.10**) with either SO_2Cl_2 or Br_2. The normal sequence of the SCOOPY reaction is reversed in that the reaction of the keto acid phosphorane (**1.06-1.10**) promotes enol-lactonization and hence formation of the β-oxido group of (**1.38**) (SCHEME 1.12) and (**1.37**) (SCHEME 1.10). Loss of triphenylphosphine oxide then occurs to give the chloro enollactones (**1.11-1.15**, SCHEME 1.12) and bromo enollactones (**1.17**, SCHEME 1.10), analogous to the vinyl halide (**1.35**, SCHEME 1.13). In the standard SCOOPY reaction the β-oxido group (**1.33**, SCHEME 1.13) is generated prior to the addition of the electrophile.

SECTION 1.7

PHOSPHONIUM SALT INTERMEDIATES

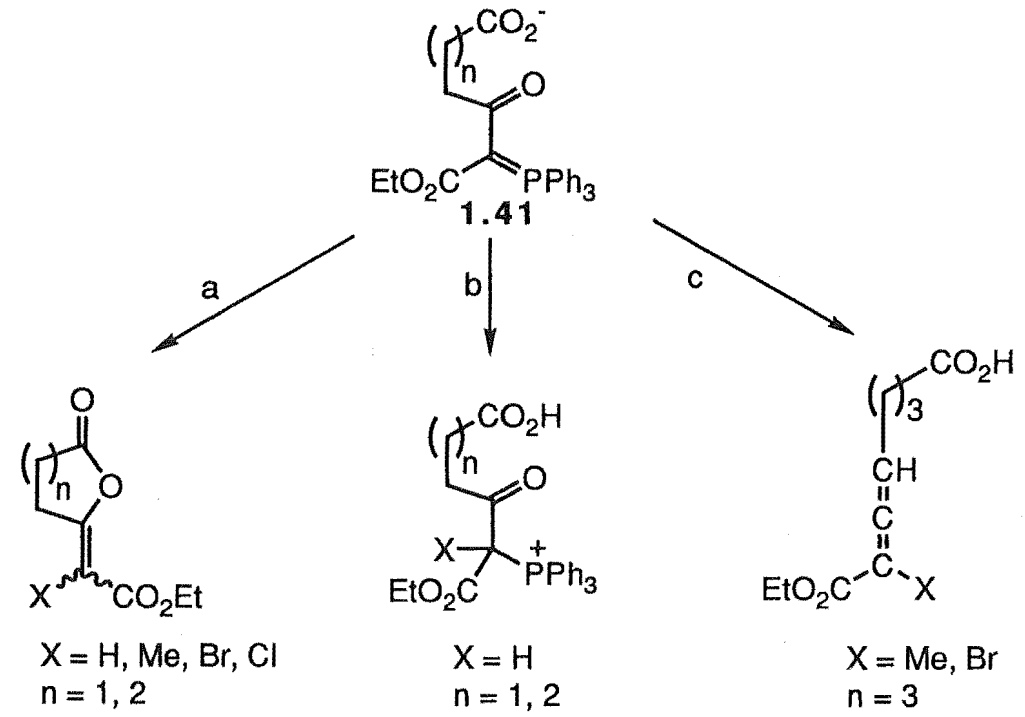
On reaction of the keto acid phosphorane (**1.06**) with Br_2 at 0 °C in $CDCl_3$, the bromo phosphonium salt intermediate (**1.39**) was detected by ^1H NMR spectroscopy[1.20]. However, similar attempts to detect the chloro phosphonium salt intermediate (**1.40**) were unsuccessful. The chloro enollactones (**1.11**) were observed to form directly from the keto acid phosphorane (**1.06**), dissolved in $CDCl_3$ at 0 °C, immediately upon addition of SO_2Cl_2.



1.39 X = Br
1.40 X = Cl

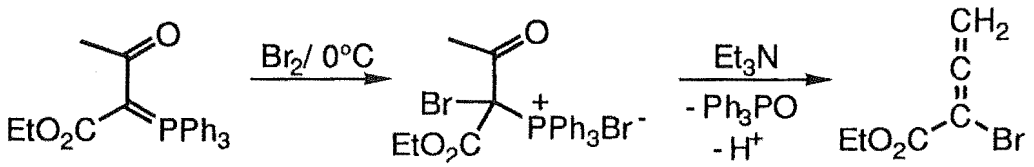
A number of different reaction pathways are available to phosphonium salts of the type (**1.41**) (SCHEMES 1.14-1.15)[1.19-1.20]. When X is H, Me, Br or Cl and n is 1 or 2 the phosphonium salt cyclizes to form enollactones (pathway a, SCHEME 1.14). When X is H and n is 1 or 2, migration of a proton can occur to give the keto acid phosphorane (pathway b, SCHEME 1.14).

SCHEME 1.14



Alternatively, enolization followed by the loss of triphenylphosphine oxide occurs to yield an allene when cyclization is not favoured, due to either a long alkyl chain (pathway c, SCHEME 1.14) or the absence of a free carboxyl group (SCHEME 1.15).

SCHEME 1.15

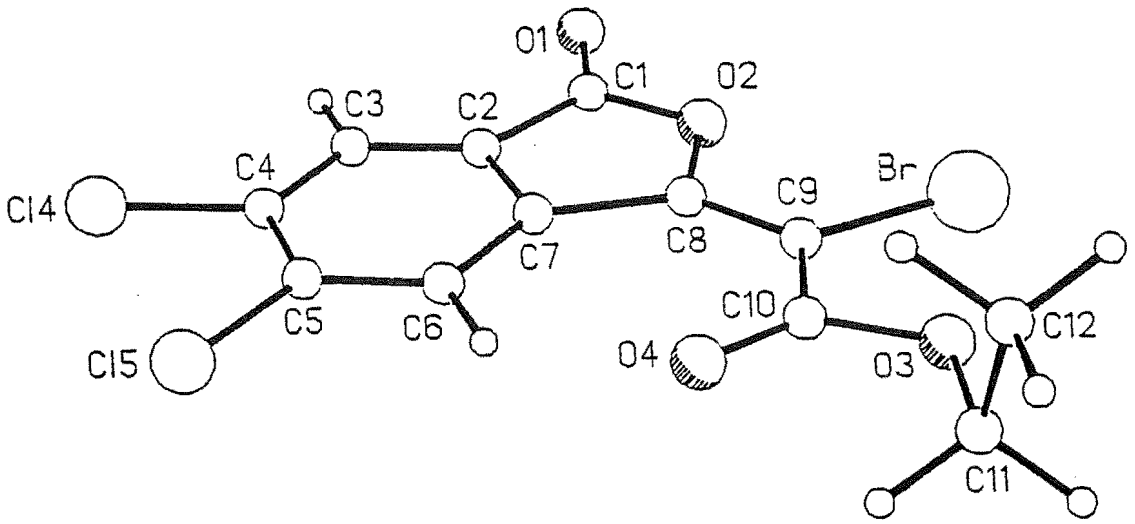


SECTION 1.8

STRUCTURE ASSIGNMENT OF BROMO AND CHLORO ENOLLACTONES

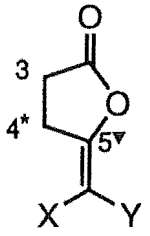
The structure of the chloro enollactones (**1.11-1.15**) was assigned by comparison of their ^{1}H and ^{13}C NMR spectra with the ^{1}H and ^{13}C NMR spectra of the corresponding bromo enollactones (**1.17-1.21**). The structure of the bromo enollactone (**1.17Z**) was determined by single crystal X-ray structure analysis and the perspective view, with crystallographic atom labelling, is shown in FIGURE 1.01.

FIGURE 1.01: Perspective View and Crystallographic Labelling of Z-Bromo Enollactone (**1.17Z**)

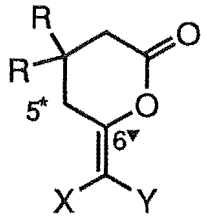


In assigning configuration, the most diagnostic resonances were $(\mathbf{H\text{-}4})_2$ and **C-5** in the succinic-based enollactones (**1.11**); $(\mathbf{H\text{-}5})_2$ and **C-6** in the glutaric-based enollactones (**1.12-1.13**); and **H-4** and **C-3** phthalic-based enollactones (**1.14-1.15, 1.17**) (TABLE 1.01). The ethyl ester in the Z configuration is known to deshield $(\mathbf{H\text{-}4})_2$, $(\mathbf{H\text{-}5})_2$ and **H-4** in succinic-, glutaric- and phthalic-based enollactones, respectively[1.21]. This trend was observed in the chloro and bromo enollactones (**1.11-1.15, 1.17-1.21**, TABLE 1.01).

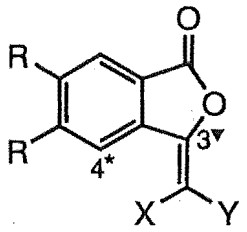
TABLE 1.01: Trends in the ^{1}H and ^{13}C NMR Spectra of Chloro and Bromo Enöllactones



	X	Y
1.11E	Cl	CO_2Et
1.11Z	CO_2Et	Cl
1.18E	Br	CO_2Et
1.18Z	CO_2Et	Br



	X	Y	R
1.12E	Cl	CO_2Et	H
1.12Z	CO_2Et	Cl	H
1.13E	Cl	CO_2Et	Me
1.13Z	CO_2Et	Cl	Me
1.19E	Br	CO_2Et	H
1.19Z	CO_2Et	Br	H
1.20E	Br	CO_2Et	Me
1.20Z	CO_2Et	Br	Me



	X	Y	R
1.14E	Cl	CO_2Et	H
1.14Z	CO_2Et	Cl	H
1.15E	Cl	CO_2Et	Cl
1.15Z	CO_2Et	Cl	Cl
1.17E	Br	CO_2Et	Cl
1.17Z	CO_2Et	Br	Cl
1.21E	Br	CO_2Et	H
1.21Z	CO_2Et	Br	H

Compd No.	E isomer δ H*	Z isomer δ H*	E isomer δ C▼	Z isomer δ C▼	Ratio E/Z [a]	Yield (E+Z)%[b]
1.11	3.14	3.43	158.5	161.6	86/14	92
1.18	3.10	3.41	158.9	162.9	70/30	77
1.12	2.83	3.20	155.4	c	96/4	73
1.19	2.80	3.16	155.2	161.0	89/11	85
1.13	2.66	3.05	154.6	c	88/12	70
1.20	2.65	3.01	154.1	159.7	88/12	86
1.14	8.49	8.72	149.7	152.9	44/56	62
1.21	8.70	8.58	149.5	153.7	35/56	55 (74)[d]
1.15	8.58	8.96	147.8	151.4	23/77	93
1.17	8.77	8.86	147.7	152.4	20/80	44 (61)[d]

a = calculated from ^{1}H NMR spectrum

b = isolated yield after chromatography

c = ^{13}C NMR spectrum ofminor isomer not recorded

d = yield of anhydride/$Ph_3P{=}CBrCO_2Et$ reaction

However, some ambiguity existed in the phthalic series due to the deshielding effect of bromine. The **H-4** resonance of the Z-halo enollactones (**1.14Z, 1.15Z, 1.17Z**) was downfield relative to the corresponding E isomers (**1.14E, 1.15E, 1.17E**) as expected. The exception was phthalic bromo enollactone (**1.21**). Z-phthalic bromo enollactone (**1.21Z**), the structure of which was confirmed by single crystal X-ray analysis, gave a resonance for **H-4** (δ 8.59) upfield relative to the corresponding E isomer (**1.21E**) (δ 8.80).

A trend observed for all bromo and chloro enollactones (**1.11-1.15, 1.17-1.21**) was that the ylidene carbon resonances were consistently downfield in the Z isomers relative to the corresponding E isomers (TABLE 1.01).

The E-halo enollactone was the major isomer for the five- and six-membered series (**1.06-1.10, 1.18-1.20**), while the Z-isomer predominated in the phthalic-based examples (**1.14-1.15, 1.17, 1.21**) (TABLE 1.01).

SECTION 1.9

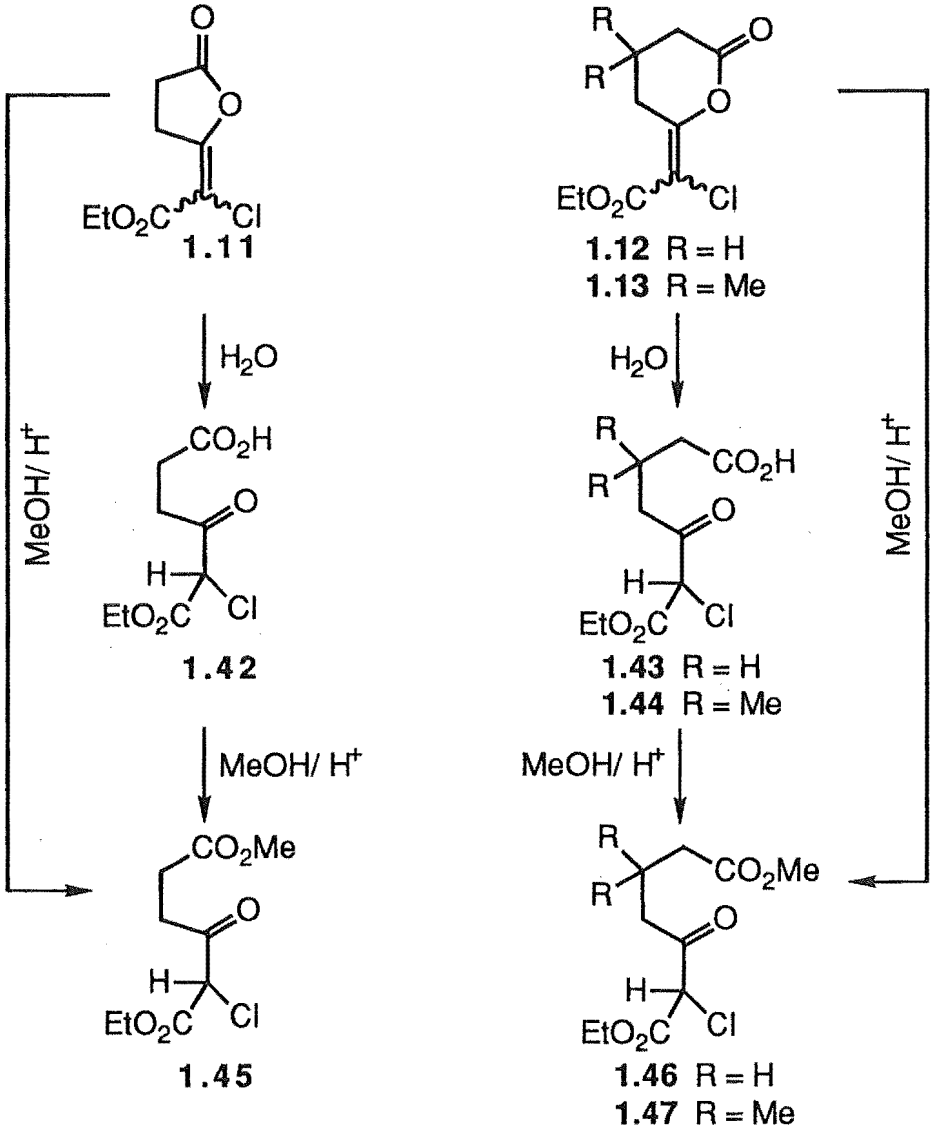
REACTIONS OF CHLORO ENOLLACTONES WITH WATER AND METHANOL

Whereas the bromo enollactones (**1.17-1.21**) were stable indefinitely at 20 °C, the succinic- and glutaric-based chloro enollactones (**1.11-1.13**) readily reacted with atmospheric H_2O, over the period of 3 weeks, to form acids (**1.42-1.44**, SCHEME 1.16).

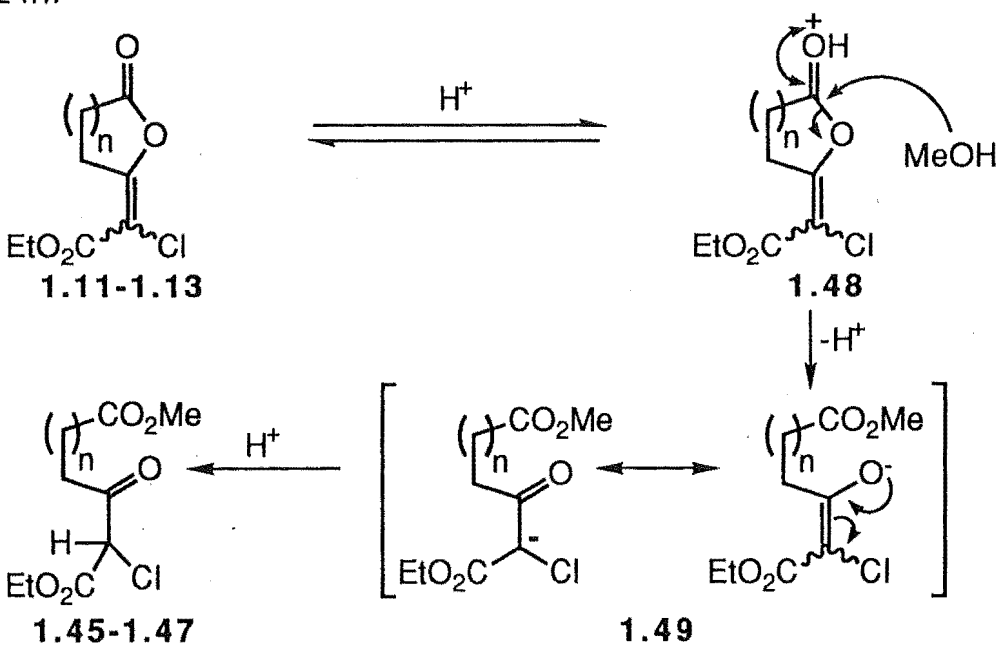
The chloro enollactones gave the methyl esters (**1.45-1.47**, SCHEME 1.17) after contact with silica gel chromatotron plates containing methanol. The methyl esters were also formed from the corresponding acids (**1.42-1.44**), on reaction with methanol and *p*-toluene sulphonic acid (PTSA), at 20 °C.

The proposed mechanism for the formation of the methyl esters (**1.45-1.47**) from chloro enollactones (**1.11-1.13**) is shown in SCHEME 1.17. An analogous mechanism is proposed for the formation of the corresponding acids (**1.42-1.44**) from the chloro enollactones (**1.11-1.13**), but with H_2O as the nucleophile.

SCHEME 1.16

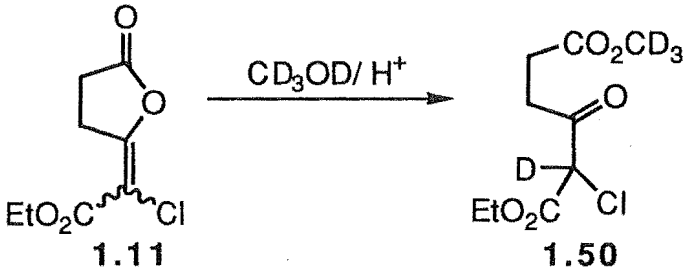


SCHEME 1.17



This mechanism was supported by the finding that reaction of succinic chloro enollactone (**1.11**) with CD_3OD in the presence of PTSA gave (**1.50**) (SCHEME 1.18), which showed characteristic deuterium (D) NMR signals for the methyl ester and **H-2** (TABLE 1.02).

SCHEME 1.18



1.11 **1.50**

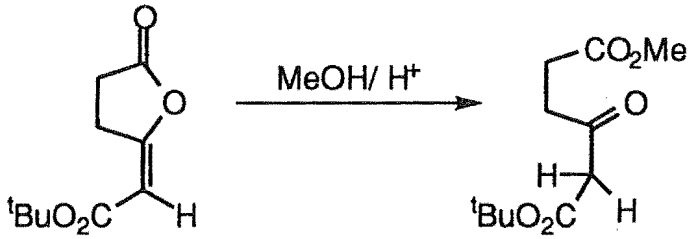
TABLE 1.02

resonance	δ H (**1.45**)	δ D (**1.50**)
CO_2CH_3	3.69	3.66
CHCl	4.87	5.09

The facile reaction of the chloro enollactones (**1.11-1.13**) with H_2O or methanol suggests that their therapeutic potential as mechanism-based inactivators is very limited. The cell environment is aqueous, hence it is unlikely that the chloro enollactones (**1.11-1.13**) would reach their target unchanged. A good mechanism-based inactivator must be stable in the cell environment and react only with the target enzyme. However, the reaction chloro enollactones (**1.11-1.13**) underwent with H_2O and methanol (SCHEME 1.16) does mimic the initial reaction required for protease mechanism-based inactivation (steps a and b, SCHEME I.02, *Section I.3.1* in the Introduction). Hence it is reasonable to assume that less reactive halo enollactones, (**1.14-1.15, 1.17**), will be capable of inactivating the target enzyme via a mechanism-based route.

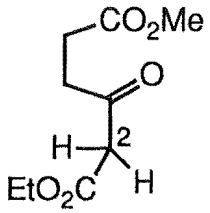
The *tert*-butyl protio enollactone (**1.51**) underwent an analogous reaction, also following contact with silica gel chromatotron plates containing methanol, to give the methyl ester (**1.52**) (SCHEME 1.19).

SCHEME 1.19



1.51 **1.52**

The acids (**1.42-1.44**) and methyl esters (**1.45-1.47**, **1.52**) gave similar ^{1}H and ^{13}C NMR spectra to **3.01** (from Chapter 3). However, as a consequence of the chiral centre at C-2 in the acids (**1.42-1.44**) and methyl esters (**1.45-1.47**), the resonance arising from $(H\text{-}4)_2$ appeared as a multiplet for (**1.42-1.44** and **1.45-1.47**), whereas **H-4** was a triplet for (**1.52** and **3.01**). The deshielding effect of Cl relative to H accounts for the significant differences between the ^{1}H and ^{13}C NMR spectra of (**1.45-1.47** and **1.42-1.44**) (compounds which contain Cl at C-2), as compared with the spectra of (**1.52** and **3.01**) (compounds which contain hydrogen at C-2) (TABLE 1.03).



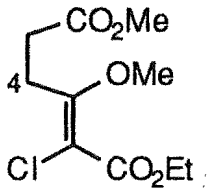
3.01

TABLE 1.03

Compd no.	δ **H-2**	δ **C-2**
1.42	4.88	63.3
1.43	4.87	63.2
1.45	4.79	63.3
1.46	4.78	67.2
1.47	4.81	a
1.52	3.40	50.6
3.01	3.50	51.7

a ^{13}C NMR spectrum was not recorded

Attempts to form the methyl ester (**1.45**) from the corresponding acid (**1.42**) by methylation with CH_2N_2 were unsuccessful. A compound, tentatively assigned as the alkene (**1.53**) was isolated.



1.53

Assignment of the product to the structure indicated by (**1.53**) was based upon spectral data. The composition of the product; *ie*, $C_{10}H_{15}ClO_5$, was consistent with the high resolution mass spectrum. The ^{13}C NMR spectrum showed no evidence of a ketone or **C**HCl group; instead there were resonances at δ 164.0 and δ 104.9 corresponding to the carbons of the double bond. Also, there were resonances at δ 52.0 and δ 56.2 indicating two new OMe groups. The ^{1}H NMR spectrum also indicated the presence of two new OMe groups and the absence of the C**H**Cl resonance. As a result, structure (**1.53**) was proposed. The configuration was assigned as E because the (**H-4**)$_2$ protons (δ 3.19) resonated at approximately the same chemical shift as the analogous (**H-4**)$_2$ protons in the E-chloro enollactone (**1.11E**) (δ 3.14). The ^{1}H and ^{13}C NMR spectra of the product after purification by radial chromatography suggested that in addition to (**1.53**), a small amount of another compound (perhaps the Z isomer of the alkene (**1.53**)) was present.

SECTION 1.10

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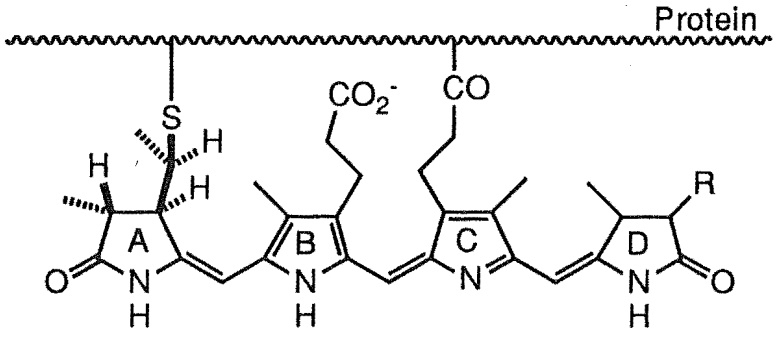
CHAPTER 2

SYNTHESIS OF ENAMINO ESTERS VIA THE INSERTION REACTION

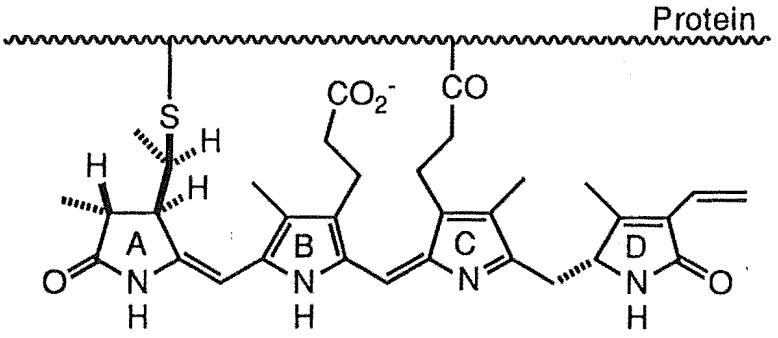
SECTION 2.1

INTRODUCTION

Enamino esters are a sub-class of the important group of compounds known as ene-lactams. Ene-lactams are found in nature. For example, biliproteins[2.01] (**2.01-2.03**) are a family of chromophores consisting of a linear tetrapyrrole covalently bonded to a protein. Rings A (**2.01-2.03**) and D (**2.01-2.02**) of the biliproteins are ene-lactams.



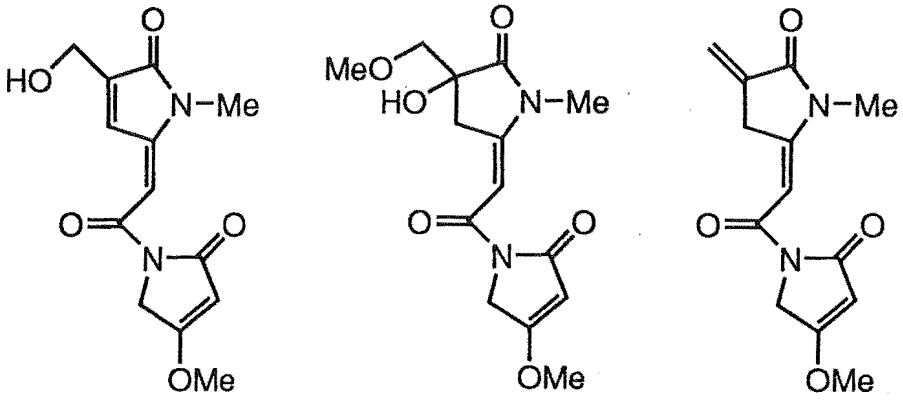
2.01 R = Et *eg* phycochromes
2.02 R = $CH{=}CH_2$ *eg* phytochromes



2.03 *eg* phycoerythrobilin

Biliproteins are found in red algae, cyanobacteria, cryptophytes, mosses and higher green plants where they are important in photosynthesis and photomorphogenesis.

Pukeleimides, exemplified by pukeleimide A (**2.04**), pukeleimide C (**2.05**) and pukeleimide E (**2.06**), is another naturally occurring class of compound containing an ene-lactam unit.



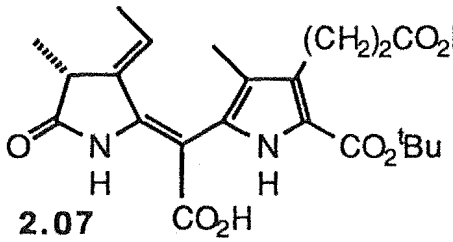
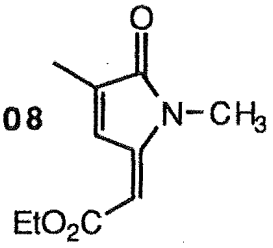
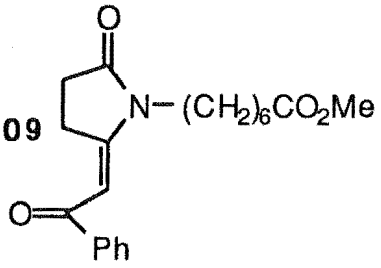
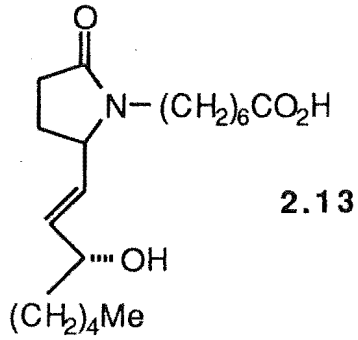
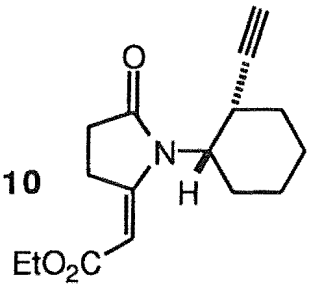
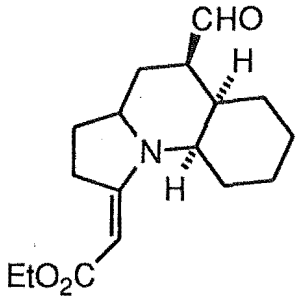
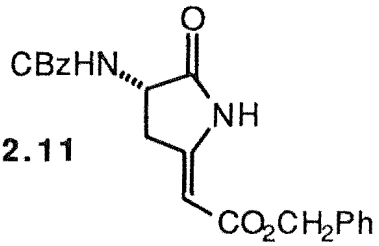
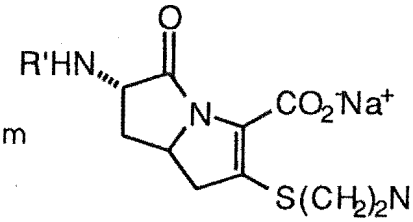
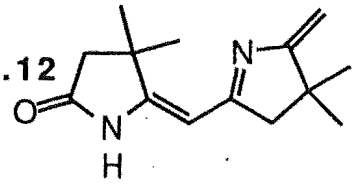
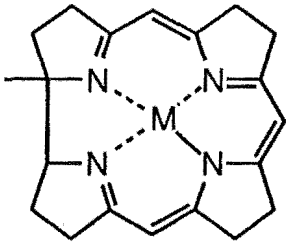
2.04 **2.05** **2.06**

Pukeleimides have been isolated from strains of the marine blue-green alga, Lyngbya majuscula responsible for the contact dermatitis known as 'swimmer's itch'[2.03].

Ene-lactams, including cyclic enamino esters, are also versatile synthetic intermediates. TABLE 2.01 depicts ene-lactams (**2.07-2.12**) which have been used as intermediates in syntheses of natural products and analogues of natural products (**2.03-2.04, 2.13-2.16**)[2.03-2.08].

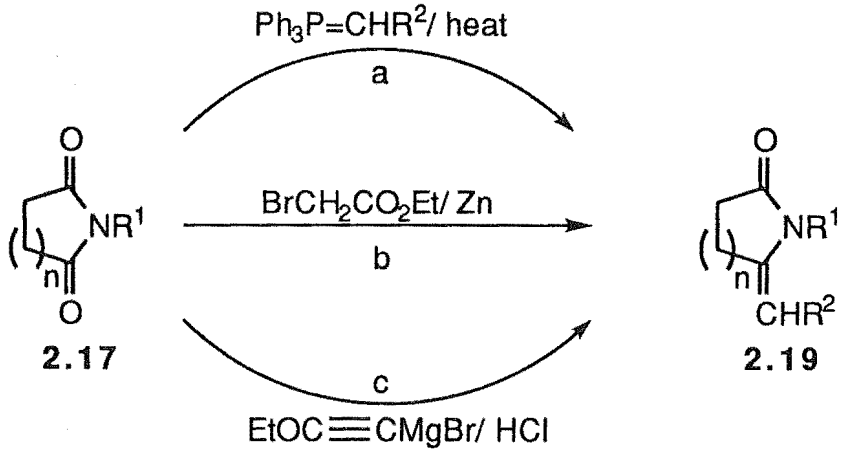
Another important application of cyclic acylated enamino esters, which is developed in this thesis, is as potential peptide analogue inhibitors of serine proteases (discussed in *Section I.5* in the Introduction).

TABLE 2.01: Enamino Esters of Synthetic Importance

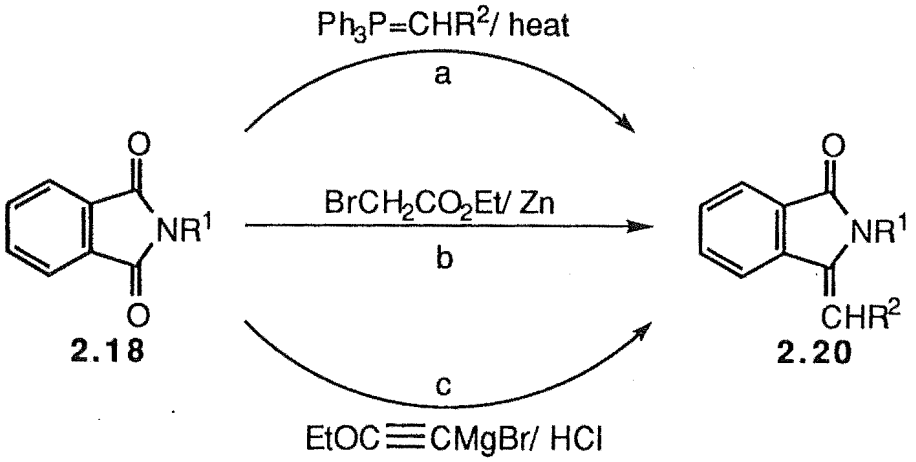
Enamino Ester Intermediate	Target Molecule	Structure of Target Molecule
 **2.07**	chromophore of biliproteins	**2.03**
 **2.08**	analogues of pukeleimide A	**2.04**
 **2.09**	prostaglandin analogue	 **2.13**
 **2.10**	gephyrotoxin analogue	 **2.14**
 **2.11**	carbapenam analogues	 **2.15**
 **2.12**	corrins (*eg* vitamin B_{12}) and corrin analogues	 **2.16**

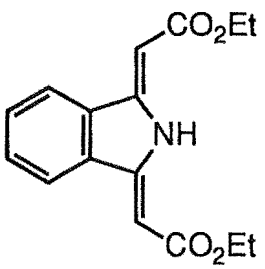
Therefore, cyclic enamino esters represent an attractive synthetic target. Traditionally, cyclic enamino esters (**2.19** and **2.20**) have been prepared from the corresponding imide (**2.17** and **2.18**, respectively) via either a Wittig[2.09-2.11] (pathway a, SCHEMES 2.01 and 2.02), Reformatsky[2.11-2.12] (pathway b, SCHEMES 2.01 and 2.02) or Grignard[2.11-2.12] reaction (pathway c, SCHEMES 2.01 and 2.02). These reactions suffer from low yields, harsh reaction conditions (TABLE 2.02) and undesirable side reactions, such as bis adduct formation (for example **2.21**), and subsequent isomerization to pyrroles (for example **2.22**).

SCHEME 2.01

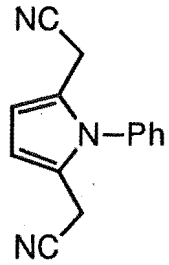


SCHEME 2.02





2.21



2.22

Synthesis of enamino esters via the Wittig, Reformatsky or Grignard reactions has essentially been limited to five-membered succinimide- and phthalimide-based systems. The synthesis of one example of a six-membered enamino ester has been reported via the Wittig methodology (Entry 5 in TABLE 2.02).

TABLE 2.02: Syntheses of Enamino Esters via Grignard, Wittig and Reformatsky Reactions

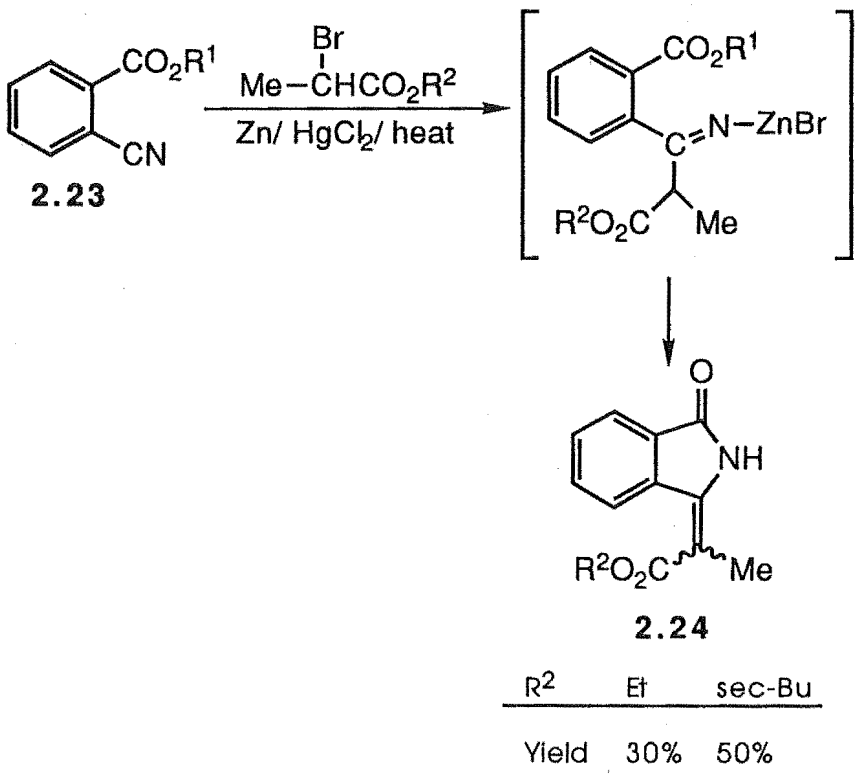
Pathway	SCHEME	n	R^1	R^2	Yield%	E vs Z	Conditions
a	2.01	1	H	Ph	13	*	xylene/ 140 °C/ 20h
a	2.01	1	Me	Ph	12	E	xylene/ 140 °C/ 20h
a	2.01	1	Me	CO_2Et	32	E	melt/ 130 °C/ 18h
a	2.01	1	Ph	CN	33	E	melt/ 200 °C/ 4h
a	2.01	2	Me	CO_2Et	11	E	melt/ 200 °C/ 4h
a	2.02	-	H	H	24	*	C_6H_6/ 80 °C/ 5h
a	2.02	-	Me	Ph	21	56E/44Z	xylene/ 140 °C/ 20h
a	2.02	-	H	CO_2Et	33	Z	melt/ 140 °C/ 4.5h
a	2.02	-	H	CN	63	Z	xylene/ 140 °C/ 24h
b	2.01	1	H	CO_2Et	24	88E/12Z	
b	2.02	-	H	CO_2Et	50	Z	
b	2.02	-	Me	CO_2Et	62	E	
c	2.01	1	Me	CO_2Et	68	E	
c	2.02	-	Me	CO_2Et	52	*	

* E/Z isomer ratio not reported

Enamino esters have also been synthesized via variations of the Wittig and Reformatsky reactions discussed above. The Reformatsky reaction of *o*-cyanobenzoic esters (**2.23**) has been used to synthesize phthalimide-based enamino esters[2,13] (**2.24**) (SCHEME 2.03). The analogous Wittig reaction of $Ph_3P{=}CHCO_2Et$ with cyclic

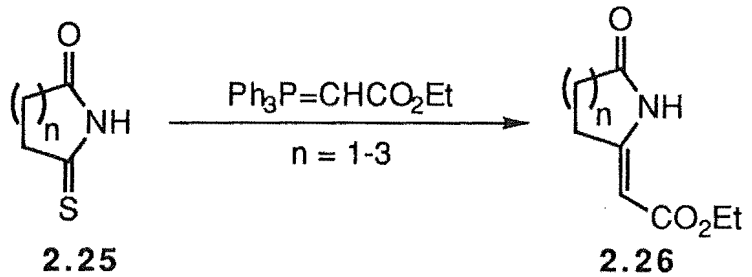
monothiodicarboximides (**2.25**) has been used to synthesize five-, six- and seven-membered enamino esters[2.14] (**2.26**) (Scheme 2.04).

SCHEME 2.03



R^2	Et	sec-Bu
Yield	30%	50%

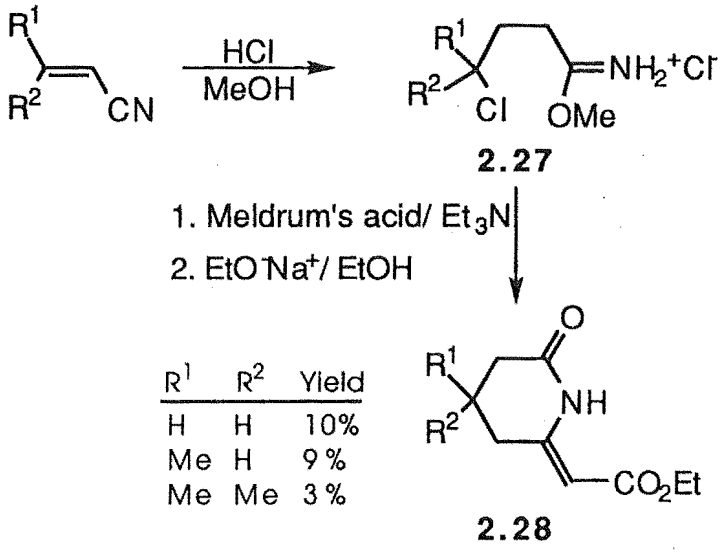
SCHEME 2.04



n	1	2	3
Yield	66%	45%	51%

Six-membered enamino esters (**2.28**) have also been prepared from imidate hydrochlorides[2.15] (**2.27**) (SCHEME 2.05), in low yields, and as for the reactions in SCHEMES 2.03 and 2.04 the substituent on N is always hydrogen.

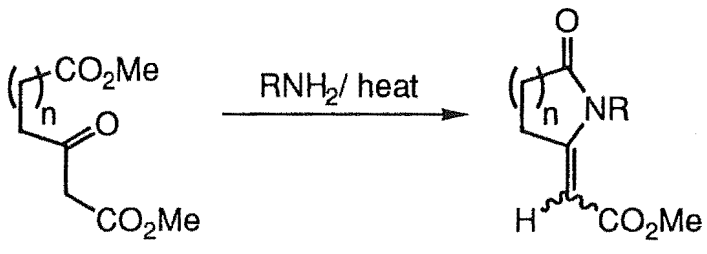
SCHEME 2.05



R^1	R^2	Yield
H	H	10%
Me	H	9%
Me	Me	3%

More recently, cyclic acylated enamino esters (**2.30** and **2.32**) have been prepared from β-keto esters[2.17] (**2.29**) (SCHEME 2.06) and by treatment of an oxirane (**2.31**) with sodium azide and $RNH_2.HCl$[2.16] (SCHEME 2.07). These methods give, at best, modest yields and lack generality. The syntheses of enamino esters described in Chapter 3 are based on the former reaction (SCHEME 2.06).

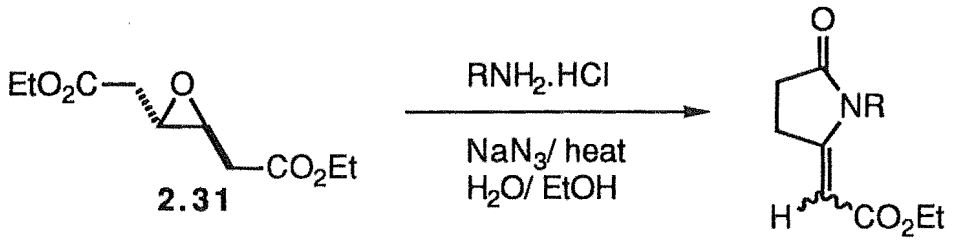
SCHEME 2.06



2.29 **2.30**

n	R	E vs Z	Yield%	Conditions
1	H	Z	56	8h/ 140 °C
1	CH_2Ph	E	62	1. 18h reflux in toluene/ 2. NaH
1	^iPr	E	35	1. 18h reflux in toluene /2. NaH
1	^nBu	E	40	1. 18h reflux in toluene/ 2. NaH
1	CH_2=$CHCH_2$	E	49	1. 18h reflux in toluene/ 2. NaH
2	CH_2Ph	E	54	1. 18h reflux in toluene/ 2. NaH

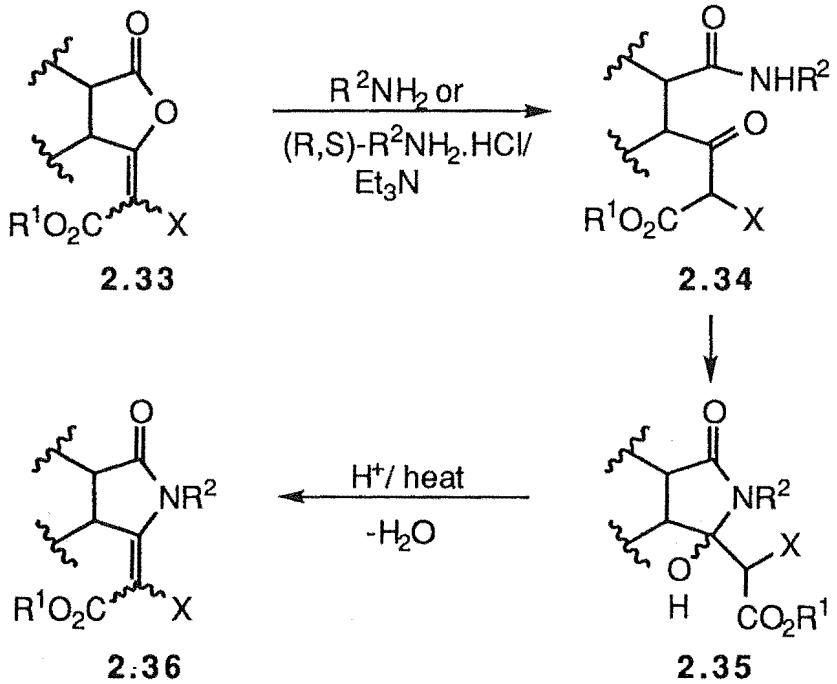
SCHEME 2.07



R	H	Me	Pr
Yield	67%	67%	62%

This chapter describes a new, versatile, high yielding and convenient synthesis of succinimide- and phthalimide-based enamino esters (SCHEME 2.08). The reaction of an enollactone (**2.33**), readily prepared via Wittig chemistry[2.10], with an amine, initially forms an isolable keto-amide (**2.34**) and/or hydroxy lacatm (**2.35**) intermediate. Subsequent elimination of H_2O on heating gives the enamino ester (**2.36**), via an overall insertion process.

SCHEME 2.08



As discussed in the Introduction (*Section i.5*), enamino esters are potential mechanism-based inactivators and alternate substrate inhibitors of serine proteases. This chapter is concerned with the synthesis of model enamino esters via the new insertion reaction (SCHEME 2.08) and the establishment of optimum conditions and limits for the insertion reaction before its application to the synthesis of the target peptide analogues (**i.34** and **i.35**, *Section i.5*, Introduction).

SECTION 2.2

KETO-AMIDES AND HYDROXY LACTAMS

SECTION 2.2.1

SYNTHESIS OF KETO-AMIDES AND HYDROXY LACTAMS

The keto-amides (**2.37-2.47**) and the hydroxy lactams (**2.48-2.50**) were prepared via the reaction of the appropriate enollactone (**2.33a-d**), synthesized using standard Wittig chemistry[2.10], with a primary amine (SCHEME 2.09). The results are summarized in TABLE 2.03.

The keto-amide (**2.37**) was obtained in 100% yield, by stirring a CH_2Cl_2 solution of succinic enollactone (**2.33a**) with a solution of NH_3 (8 equivalent) in ethanol. After 5h the solvent was evaporated under reduced pressure to yield keto-amide (**2.37**). An identical method using succinic enollactone (**2.33a**) and NH_3 (11 equivalent) in ethanol, quantitatively gave hydroxy lactam (**2.48**).

Keto-amides (**2.38-2.41**) and hydroxy lactam (**2.49**) were prepared, also in 100% yield, via reaction of the appropriate enollactone (**2.33a-2.33b, 2.33d**) with methyl-, ethyl-, or ^{n}butyl-amine (1 or 1.8 equivalent) in CH_2Cl_2 at 20 °C for 16h (TABLE 2.03).

Keto-amides (**2.42-2.47**) and hydroxy lactam (**2.50**) were prepared, in yields of 73-100%, via the reaction of the appropriate enollactone (**2.33a-2.33d**) with (R,S)-alanine-, (R,S)-leucine-, (R,S)-phenylalanine-, or glycine-ethylester hydrochloride, and triethylamine, in CH_2Cl_2 at 20 °C for 16h (TABLE 2.03).

SCHEME 2.09

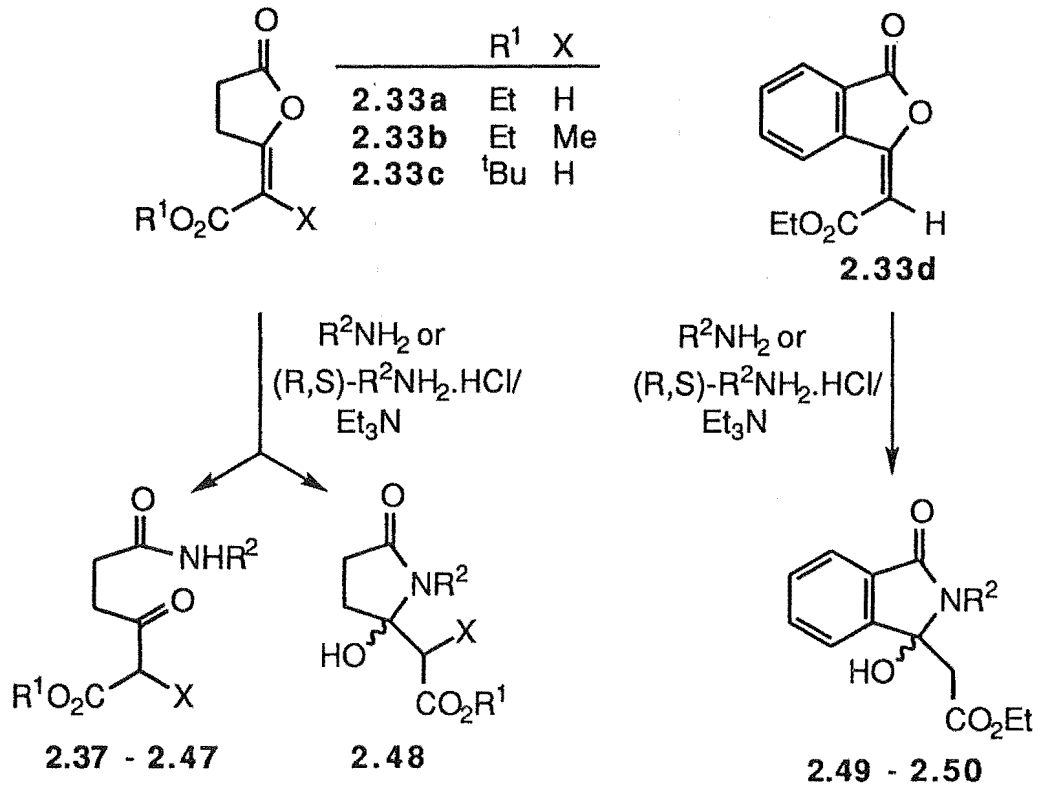
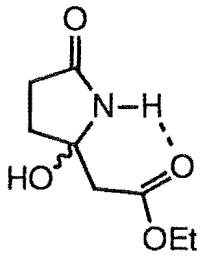


TABLE 2.03

Compd	X	R^1	R^2	Yield%	Equiv. Amine
2.37	H	Et	H	100	8
2.38	H	Et	Me	100	11
2.39	H	Et	Et	100	1.8
2.40	H	Et	^{n}Bu	100	1
2.41	Me	Et	^{n}Bu	100	1
2.42	H	Et	(R,S)-CH(Me)CO_2Et	73	1.3
2.43	H	Et	(R,S)-CH(CH_2CHMe_2)CO_2Et	100	1.3
2.44	H	Et	(R,S)-CH(CH_2Ph)CO_2Et	97	1.3
2.45	H	Et	CH_2CO_2Et	88	1.3
2.46	H	^{t}Bu	CH_2CO_2Et	87	1
2.47	Me	Et	CH_2CO_2Et	88	1.2
2.48	H	Et	H	100	11
2.49			^{n}Bu	100	1.3
2.50			CH_2CO_2Et	79	1.4

The hydroxy lactams (**2.48-2.50**) were isolated, rather than the corresponding keto-amides, following reaction of succinic enollactone (**2.33a**) with an excess of NH_3 and after reaction of phthalic enollactone (**2.33d**) with butylamine and glycine ethylester hydrochloride. A stable six-membered ring with intramolecular hydrogen-bonding between the amino H and the carbonyl O (SCHEME 2.10) might account for the formation of hydroxy lactam (**2.48**). Formation of the phthalimide-based hydroxy lactams (**2.49-2.50**) may be a consequence of the aromatic ring in the corresponding keto-amide precursors holding the ketone and amide substituents in an orientation favourable to cyclization.

SCHEME 2.10



2.48

SECTION 2.2.2

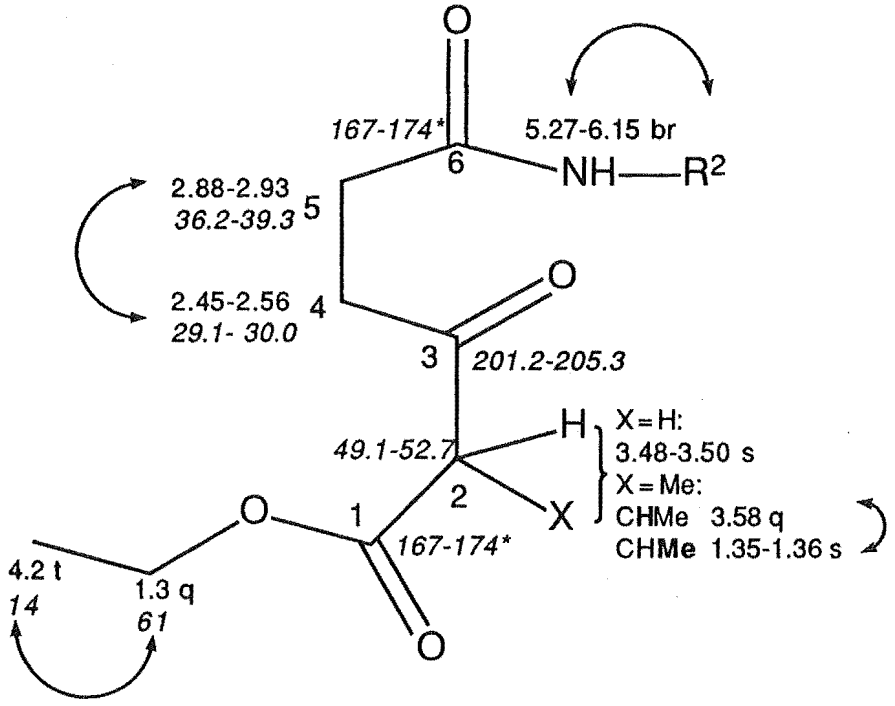
STRUCTURE ASSIGNMENT OF KETO-AMIDES AND HYDROXY LACTAMS

The structure of the keto-amides (**2.37-2.47**) and hydroxy lactams (**2.48-2.50**) was assigned primarily on the basis of ^1H NMR, ^{13}C NMR and IR spectroscopic and high resolution mass spectrometric data.

KETO-AMIDES (**2.37-2.47**)

SCHEME 2.11 summarizes the ^1H and ^{13}C NMR assignments for the keto-amides (**2.37-2.47**). For keto-amides (**2.37-2.40, 2.45-2.46**; X = H) the $(\mathbf{H\text{-}4})_2$ and $(\mathbf{H\text{-}5})_2$ resonances appeared as triplets, while for keto-amides with X=Me (**2.41** and **2.47**) the $(\mathbf{H\text{-}4})_2$ protons are diastereotopic and appeared as a multiplet. For the amino acid derived keto-amides (**2.42-2.44**), the diastereotopic $(\mathbf{H\text{-}5})_2$ protons appeared as a multiplet. These observations were used to assign the **H-4** and **H-5** resonances.

SCHEME 2.11: Range of ^{1}H (plain text) and ^{13}C (*italicized text*) NMR data in ppm for keto-amides (**2.37-2.47**)



NB *Tert*-butyl keto-amide (**2.46**) also fits this pattern except (**H-2**)$_2$ δ 3.41.

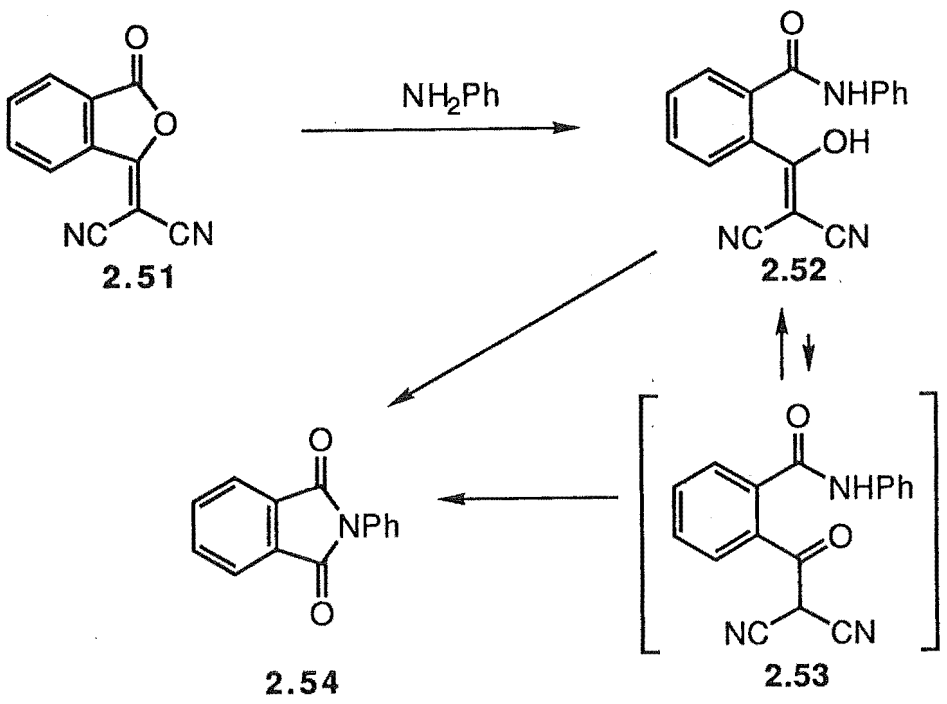
* No differentiation was made between the amide and ester carbonyl.

Arrows denote coupling observed between designated proton resonances.

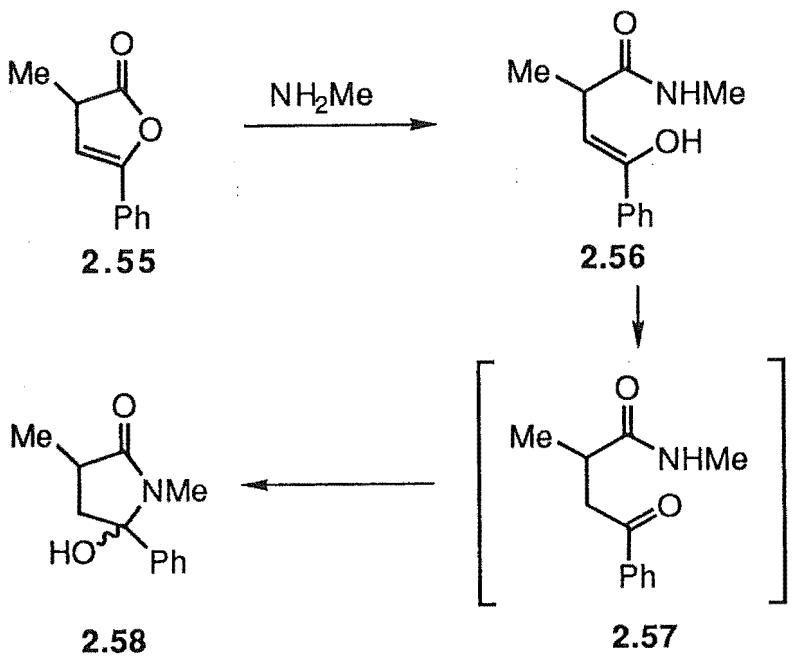
The IR and high resolution mass spectra were also consistent with the keto-amide structures (**2.37-2.47**). Further, for keto-amide (**2.39**) recrystallization was possible and the sample thus obtained was determined, by combustion analysis, to have the expected % C, H and N. Combustion analyses were not obtained for keto-amides (**2.37-2.38**, **2.40-2.47**) because the corresponding enamino esters readily formed on distillation or recrystallization.

To the best of our knowledge the keto-amides (**2.37-2.47**) have not previously been isolated or characterized. However, related keto-amides (**2.53** and **2.57**) have been postulated as reaction intermediates in the synthesis of imide (**2.54**) from pseudoanhydride[2.18] (**2.51**) (SCHEME 2.12) and in the synthesis of hydroxy lactam (**2.58**) from lactone[2.19] (**2.55**) (SCHEME 2.13).

SCHEME 2.12



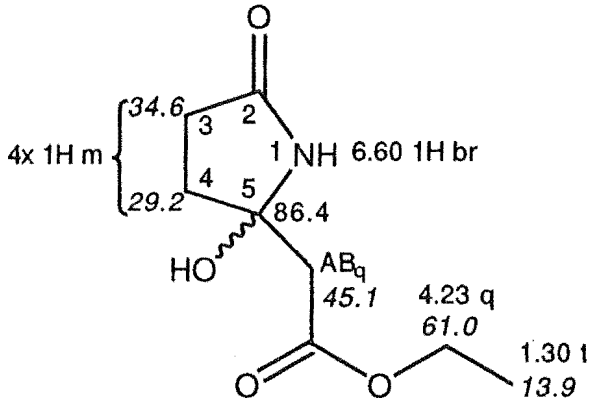
SCHEME 2.13



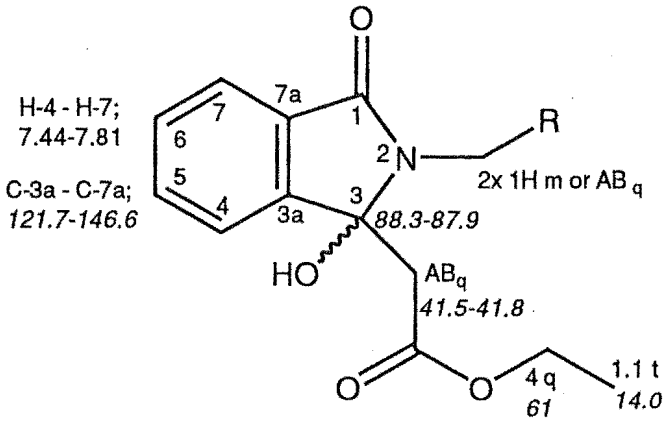
HYDROXY LACTAMS (**2.48-2.50**)

The ^{1}H and ^{13}C NMR data of the hydroxy lactams (**2.48-2.50**), summarized in SCHEME 2.14, were also characteristic. For succinimide-based hydroxy lactam (**2.48**) an increase in complexity relative to keto-amides (**2.37-2.47**) was observed for the resonances arising from CH_2CO_2Et, $(H\text{-}3)_2$ and $(H\text{-}4)_2$, reflecting the introduction of a chiral centre at C-5. In addition, the NH resonance integrated for 1 proton whereas in the corresponding keto-amide (**2.34**) the analogous resonance integrated for 2 protons.

SCHEME 2.14: Summary of ^{1}H (plain text) and ^{13}C (*italicized text*) NMR Data of Hydroxy lactams (**2.48-2.50**)



2.48



2.49-2.50

The major differences between the ^{1}H NMR spectra of the keto-amides (**2.37-2.47**) and the phthalimide-based hydroxy lactams (**2.49-2.50**) were the absence of a NH resonance and the increase in complexity of the resonances for NCH_2 (2x 1H multiplets in

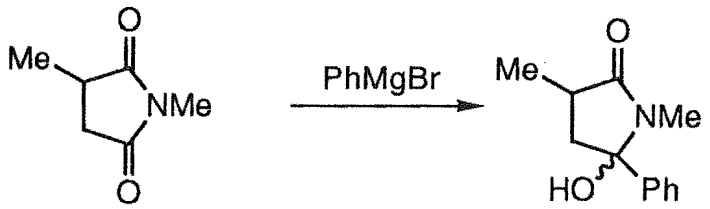
(**2.49**) and AB_q in (**2.50**)) and CH_2CO_2Et (AB_q) , reflecting the introduction of a chiral centre at C-3.

The most characteristic resonances in the ^{13}C NMR spectra were those arising from **C**-OH; **C-5** in the succinimide-based hydroxy lactam (**2.48**) and **C-3** in the phthalimide-based hydroxy lactams (**2.49-2.50**), at δ 86.4, 88.3 and 87.9, respectively and those arising from CH_2CO_2Et at δ 45.1, 41.5, 40.4, respectively. The ^{13}C NMR spectra also indicated that (**2.48-2.50**), unlike the keto-amides (**2.37-2.47**), did not contain a ketone functionality because a resonance was not observed at δ ~200.

The IR and high resolution mass spectra were also consistent with the hydroxy lactam structures (**2.48-2.50**).

Hydroxy lactams are known compounds. For example, hydroxy lactam (**2.58**) has been prepared from reaction of PhMgBr with an imide[2.19] (**2.59**) (SCHEME 2.15) and from lactone (**2.55**) and NH_2Me [2.19] (SCHEME 2.13).

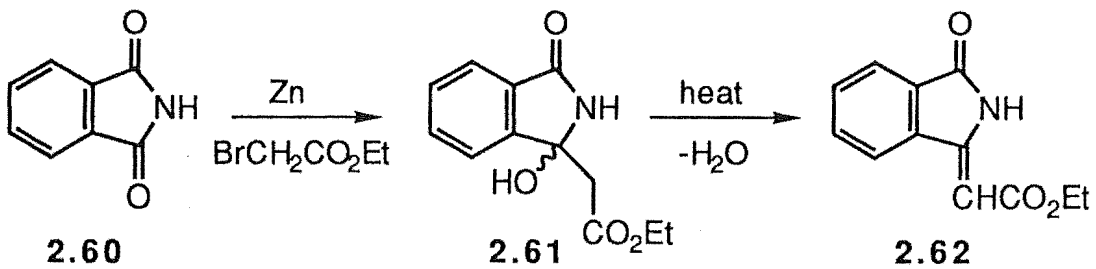
SCHEME 2.15



2.59 **2.58**

The Reformatsky reaction of phthalimide (**2.60**) with bromo acetic acid ethylester was used to prepare the hydroxy lactam (**2.61**), which dehydrated to the corresponding enamino ester (**2.62**) on heating[2.12] (SCHEME 2.16).

SCHEME 2.16



2.60 **2.61** **2.62**

SECTION 2.3

ENAMINO ESTERS

SECTION 2.3.1

SYNTHESIS OF ENAMINO ESTERS VIA THE INSERTION REACTION

The enamino esters (**2.63-2.75**) were prepared via three different methods, the results of which are summarized in TABLE 2.04:

Method A : Keto-amide (**2.37-2.47**) or hydroxy lactam (**2.48-2.50**) and *p*-toluene sulphonic acid (PTSA) were refluxed in 1, 2-dichloroethane for 3-43h, with azeotropic removal of H_2O. The solution was washed with H_2O to remove PTSA, dried ($MgSO_4$) and evaporation of the solvent under reduced pressure yielded enamino ester (**2.63-2.75**) which was purified by recrystallization or distillation. In the case of the methyl enamino esters (**2.67, 2.73**) purification was achieved by radial chromatography.

Method B : As for Method A except that the solvent was benzene.

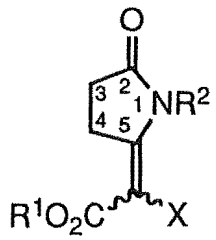
Method C : A 1, 2-dichloroethane solution of enollactone (**2.33a**) and methyl-, ethyl-, or ^{n}butyl-amine, containing activated 4Å sieves, was heated at 65 °C for 3-4 days. The solution was filtered to remove the sieves and the solvent was evaporated under reduced pressure to yield the enamino ester which was purified by recrystallization or distillation.

Problems were encountered in the synthesis of *tert*-butyl enamino ester (**2.72**) due to acid catalyzed hydrolysis of the *tert*-butyl group. Pure enamino ester (**2.72**) was obtained following radial chromatography of the corresponding keto-amide (**2.46**); however, the yield was low; 23%.

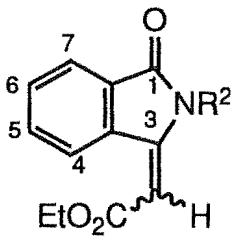
Method C represents a direct one step synthesis of enamino esters from enollactones and amines. However, Method C was not generally applicable. For example; attempted syntheses of enamino esters (**2.67** and **2.75**) via Method C led to the isolation of the keto-amide (**2.41**) and hydroxy lactam (**2.49**), respectively, even after heating for 6 days. Similarly, when keto-amides (**2.42-2.47**) and hydroxy lactam (**2.50**) were subjected to the conditions of Method C little or no enamino ester formation

occurred. Method A was the most applicable and consistently gave the best yields. Method B was more applicable than Method C, but Method C gave superior yields (TABLE 2.04).

TABLE 2.04: Synthesis of Enamino Esters (**2.63-2.75**)



2.63 - 2.73



2.74 - 2.75

Compd	X	R^1	R^2	Cyclizn. Method	Time	Yield%	E/Z
2.63	H	Et	H	C*	3days	83	5/95
				B	2h	13	5/95
2.64	H	Et	Me	C	4days	93	100/0
2.65	H	Et	Et	B	3h	71	100/0
				C	3days	71	95/5
2.66	H	Et	^{n}Bu	C	3days	100	95/5
				C	6days	100	100/0
2.67	Me	Et	^{n}Bu	A	10h	59	84/16
				B	4h	23%	82/18
2.68	H	Et	(R,S)-CH(Me)CO_2Et	A	24h	84	100/0
2.69	H	Et	(R,S)-CH(CH_2CHMe_2)CO_2Et	A	20h	84	100/0
2.70	H	Et	(R,S)-CH(CH_2Ph)CO_2Et	A	24h	86	100/0
				B	6h	23	100/0
2.71	H	Et	CH_2CO_2Et	B	2h	83	100/0
2.72	H	^{t}Bu	CH_2CO_2Et	▼	N/A	23	100/0
2.73	Me	Et	CH_2CO_2Et	A	10h	57	74/26
				B	6h	15	86/14
2.74			^{n}Bu	A	3h	76	86/14
				B	3h	23	95/5
2.75			CH_2CO_2Et	A	3h	90	60/40

* Method C conditions with hydroxy lactam (**2.48**).

▼ Enamino ester (**2.72**) formed from keto-amide (**2.46**) during radial chromatography.

In general, the yields of enamino esters, synthesized via Methods A and C, were superior to yields of enamino esters synthesized via other literature routes (*Section 2.1*). For comparison, the NH_3-derived enamino ester (**2.63**) has been obtained via the β-keto ester (SCHEME 2.06), Wittig (pathway a, SCHEME 2.01) and Reformatsky (pathway b, SCHEME 2.01) reactions in yields of 67%[2.16], 32%[2.11] and 21%[2.11], respectively. Using the insertion reaction the NH_3-derived enamino ester (**2.63**) was synthesized in 83% yield. Also, the methylamine-derived enamino ester (**2.64**) which was prepared via the β-keto ester reaction in 67% yield[2.16], was formed in 93% yield via the insertion reaction.

SECTION 2.3.2

CHARACTERIZATION OF ENAMINO ESTERS

STRUCTURE ASSIGNMENT

The NH_3- and methylamine-derived enamino esters (**2.63** and **2.64**, respectively) prepared in the current study were identical by ^1H NMR, ^{13}C NMR and IR spectroscopy, high resolution mass spectrometry and melting point to enamino esters (**2.63** and **2.64**) reported in the literature[2.16, 2.11]. The remaining compounds (**2.65-2.75**) were assigned as enamino esters on the basis of ^1H NMR, ^{13}C NMR and IR spectroscopy, high resolution mass spectrometry and combustion analysis results consistent with known enamino esters[2.11-2.12, 2.16-2.17]. The chemical shifts of key ^1H and ^{13}C NMR resonances of enamino esters (**2.63-2.75**) are summarized in TABLE 2.05.

E/Z CONFIGURATION

The E isomer was obtained as the major isomer in all examples studied (TABLE 2.04) except in the case of the NH_3-derived enamino ester (**2.63**) (discussed later). ^1H NMR spectroscopy was used to assign the E/Z configuration of the cyclic enamino esters (**2.63-2.75**). The most diagnostic resonances were (**H-4**)$_2$ and =C**H** for the succinimide-based enamino esters (**2.63-2.73**) and **H-4** and =C**H** for the phthalimide-based enamino esters (**2.74-2.75**) (TABLE 2.05).

TABLE 2.05: Characteristic ^{1}H and ^{13}C NMR data of Enamino Esters (**2.63-2.75**)

Succinimide-Based Enamino Esters

Compd. + Confign	δ **(H-3)$_2$**, m	δ **(H-4)$_2$**, m	δ =C**H**, t or =C**Me**, t	J (Hz) =CH or =CMe	δ =C**H** or =C**Me**	δ **C-5**
2.63 E *			5.30	2.0		
2.63 Z	2.52	2.87	5.00	1.5	90.2	157.4
2.64 E	2.57	3.24	5.19	1.9	91.6	161.1
2.65 E	2.55	3.23	5.23	1.9	91.2	159.5
2.65 Z *			5.02	1.5		
2.66 E	2.55	3.23	5.21	1.9	91.2	159.8
2.66 Z *			5.02	1.5		
2.67 E	2.47	3.13	2.06	1.2	101.4	153.1
2.67 Z	2.51	2.64	1.91	1.1	101.2	143.8
2.68 E	2.59	3.28	5.10	2.0	92.4	157.8
2.69 E	2.60	3.27	5.10	1.9	92.8	157.9
2.70 E	2.46	3.13	5.11	1.9	92.7	158.0
2.71 E	2.64	3.31	5.05	2.0	92.1	159.0
2.72 E	2.62	3.28	4.98	2.0	94.0	157.7
2.73 E	2.56	3.22	1.97	1.4	101.9	152.7
2.73 Z	2.59	2.77	1.89	1.0	101.6	146.1

* Insufficient amount of the minor isomer present to obtain all ^{1}H and ^{13}C NMR spectral data

Phthalimide-Based Enamino Esters

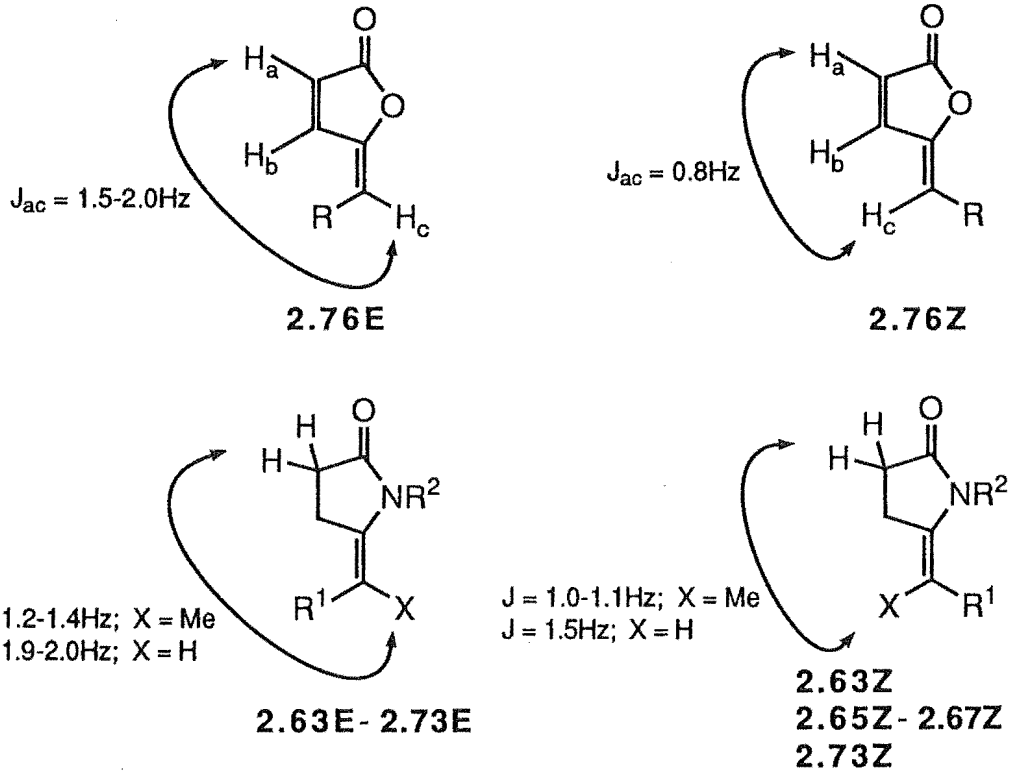
Compd	δ **H-4**	δ =C**H**	δ =**C**	δ **C**-3
2.74E	9.06	5.71	98.2	148.0
2.74Z	< 9.06	5.88	93.8	143.9
2.75E	9.10	5.54	98.7	147.7
2.75Z	7.86-7.89	5.92	94.6	144.4

The ethyl ester (or *tert*-butyl ester) in the E configuration is known to deshield **(H-4)$_2$** in succinimide-based enamino esters[2.17] and **H-4** in phthalimide-based enamino esters[2.11]. Therefore, the major isomer of the succinimide- and phthalimide-based enamino esters (**2.67**, **2.73** and **2.74-2.75**, respectively) was assigned the E configuration on the basis of the downfield shift of **(H-4)$_2$** and **H-4**, respectively, relative to the

corresponding Z isomer. For the methyl enamino esters (**2.67**, **2.73**) the assignment was confirmed by the observation of an n.O.e. between NCH_2 and $=CCH_3$ for the E isomer and an n.O.e. between $(H\text{-}4)_2$ and $=CCH_3$ for the Z isomer.

It has been reported for enol-γ-lactones that $=H_c$ of the E isomer (**2.76E**) resonates at lower field and shows a larger coupling constant, J_{ac}, relative to the corresponding Z isomer[2.20] (**2.76Z**) (SCHEME 2.17).

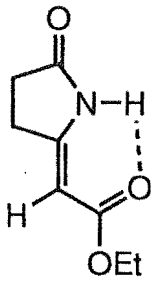
SCHEME 2.17



The same trend was observed in the methyl enamino esters (**2.67**, **2.73**); the $=CCH_3$ resonance was downfield for the E isomer (**2.67E** and **2.73E**) and showed a larger coupling constant relative to the corresponding Z isomer (**2.67Z** and **2.73Z**, respectively). The assignment of configuration to protio enamino esters (**2.63**, **2.65-2.66**) for which ^1H NMR data was obtained for both isomers, was therefore based on a comparison of =C**H** resonances. Assignment of the major isomer of (**2.63**) as the Z isomer was confirmed by the downfield position of N**H**, consistent with hydrogen-bonding (SCHEME 2.18), relative to the E isomer.

The remaining enamino esters (**2.64, 2.68-2.72**) were assigned the E configuration on the basis of the chemical shifts for the (**H-4**)$_2$ resonances, which reflected the deshielding effect of CO_2Et, and the =CH coupling constants; J=1.9-2.0Hz, which were identical to the analogous coupling constants of E enamino esters (**2.63, 2.65-2.66**) (TABLE 2.05).

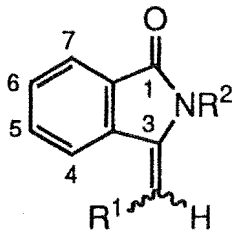
SCHEME 2.18



2.63

In contrast to the succinimide-based enamino esters (**2.63-2.73**), the resonance arising from =C**H** was upfield in the E isomer relative to the corresponding Z isomer for phthalimide-based enamino esters (**2.74-2.75**) (TABLE 2.05). This is consistent with the trend observed in literature enamino esters (**2.77-2.79**, TABLE 2.06).

TABLE 2.06



	R^1	R^2	δ=C**H** E isomer	δ=C**H** Z isomer
2.77	CO_2Et	H	5.80	6.14
2.78	CO_2Et	Me	5.30	5.57
2.79	CHO	Me	5.94	6.12

A trend observed in the ^{13}C NMR spectra of E- and Z-enamino esters (**2.67, 2.73** and **2.74-2.75**) was that the carbons of the double bond, **C=C**, were downfield in the E isomer relative to the corresponding Z isomer (TABLE 2.05). This is analogous to the trend observed in the ^{13}C NMR spectra of chloro and bromo enollactones (**1.11-1.15, 1.17-1.21**) in which the double bond carbons were consistently downfield in the Z isomers relative to the corresponding E isomers (*Section 1.8*, Chapter 1).

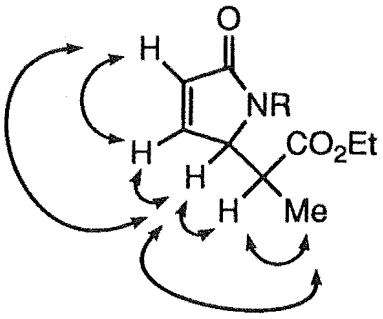
The enamino esters synthesized via the insertion reaction showed the same trends in configuration (TABLE 2.05) as enamino esters synthesized via literature routes[2.09-2.12, 2.14, 2.16-2.17]. The Z isomer predominated in the NH_3-derived enamino esters (**2.63**), possibly due to intramolecular hydrogen-bonding between NH and the ester carbonyl (SCHEME 2.18). Generally, exclusively the E isomer was formed in the succinic-derived protio enamino esters (**2.64-2.66, 2.68-2.72**). Exceptions arose with enamino ester formation via Method C, when less than 5% of the Z isomer was observed for (**2.64-2.65**), reflecting the mild conditions of this method. In the phthalimide-based enamino esters (**2.74-2.75**) and methyl enamino esters (**2.67, 2.73**), the E isomer predominated, but a significant amount of the Z isomer also formed. It was possible to separate E and Z isomers of the methyl enamino esters (**2.67, 2.73**) by radial chromatography and the E isomer of the phthalimide-based enamino ester (**2.74**) was isolated by recrystallization. For the phthalimide-based enamino ester (**2.75**) the ratio of E and Z isomers changed on heating at 60 °C for 3h, with PTSA in $CDCl_3$, from 6 E : 4 Z to 7 E : 3 Z, as determined by ^1H NMR spectroscopy.

SECTION 2.4

SIDE PRODUCTS OF THE INSERTION REACTION TO METHYL ENAMINO ESTERS

With the exception of the methyl enamino esters (**2.67, 2.73**), the enamino esters (**2.63-2.75**) were formed cleanly (as seen by the ^1H and ^{13}C NMR spectra). Purification of the glycine-derived methyl enamino ester (**2.73**) by radial chromatography also led to isolation of a compound which was assigned as the endocyclic isomer (**2.80**).

Assignment was based on the ^1H and ^{13}C NMR spectra and the results of ^1H NMR decoupling experiments (the couplings observed are indicated by the arrows). The endocyclic isomer (**2.80**) was present, by ^1H NMR spectroscopy, as a 1 : 1 mixture of diastereoisomers. After 3 days at 20 °C one diastereoisomer gave rise to E- and Z-enamino esters (**2.73**).

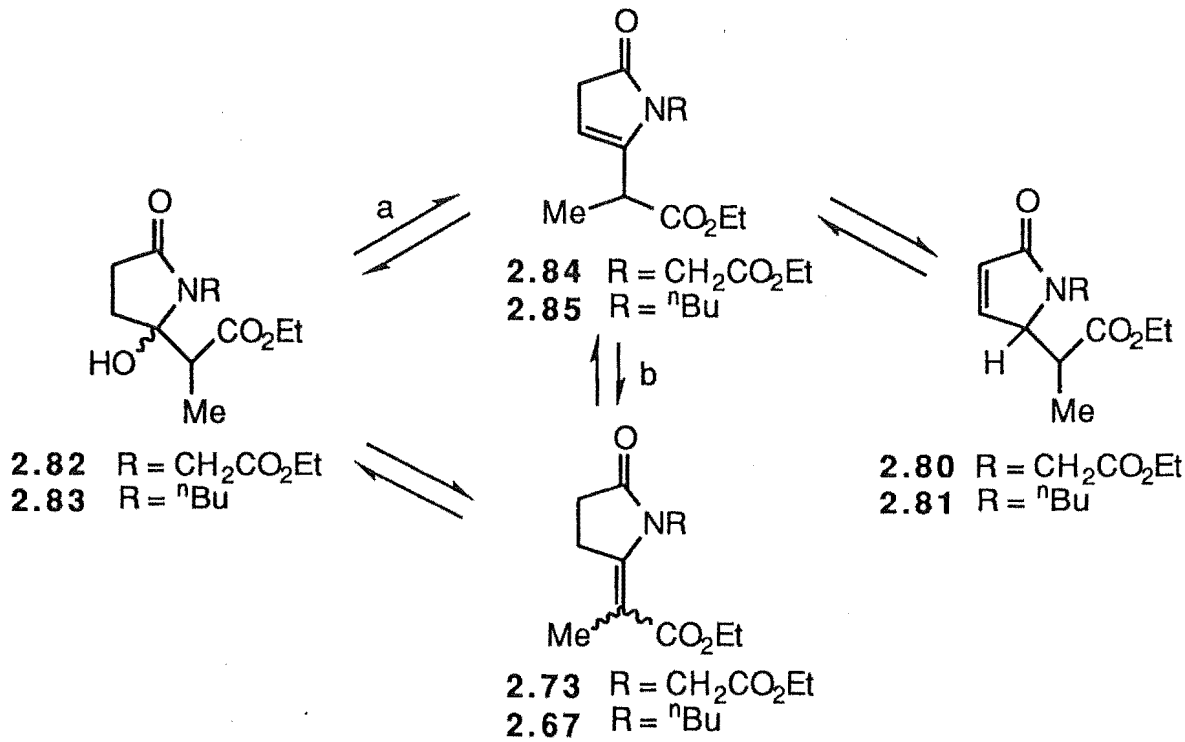


2.80 R = CH_2CO_2Et
2.81 R = ^nBu

An analogous compound (**2.81**) present in the ^1H NMR spectrum of the ^{n}butyl enamino ester (**2.67**) before radial chromatography was not isolated.

The endocyclic isomers (**2.80** and **2.81**) may form from the E- and Z-enamino esters (**2.73** and **2.67**, respectively) (pathway b, SCHEME 2.19) or from the hydroxy lactams (**2.82** and **2.83**, respectively), via (**2.84**) and (**2.85**) (pathway a, SCHEME 2.19). A related reaction was observed for another class of enamino esters whereby the reaction occurred from the enamino esters (discussed later in this chapter; *Section 2.7.1*).

SCHEME 2.19

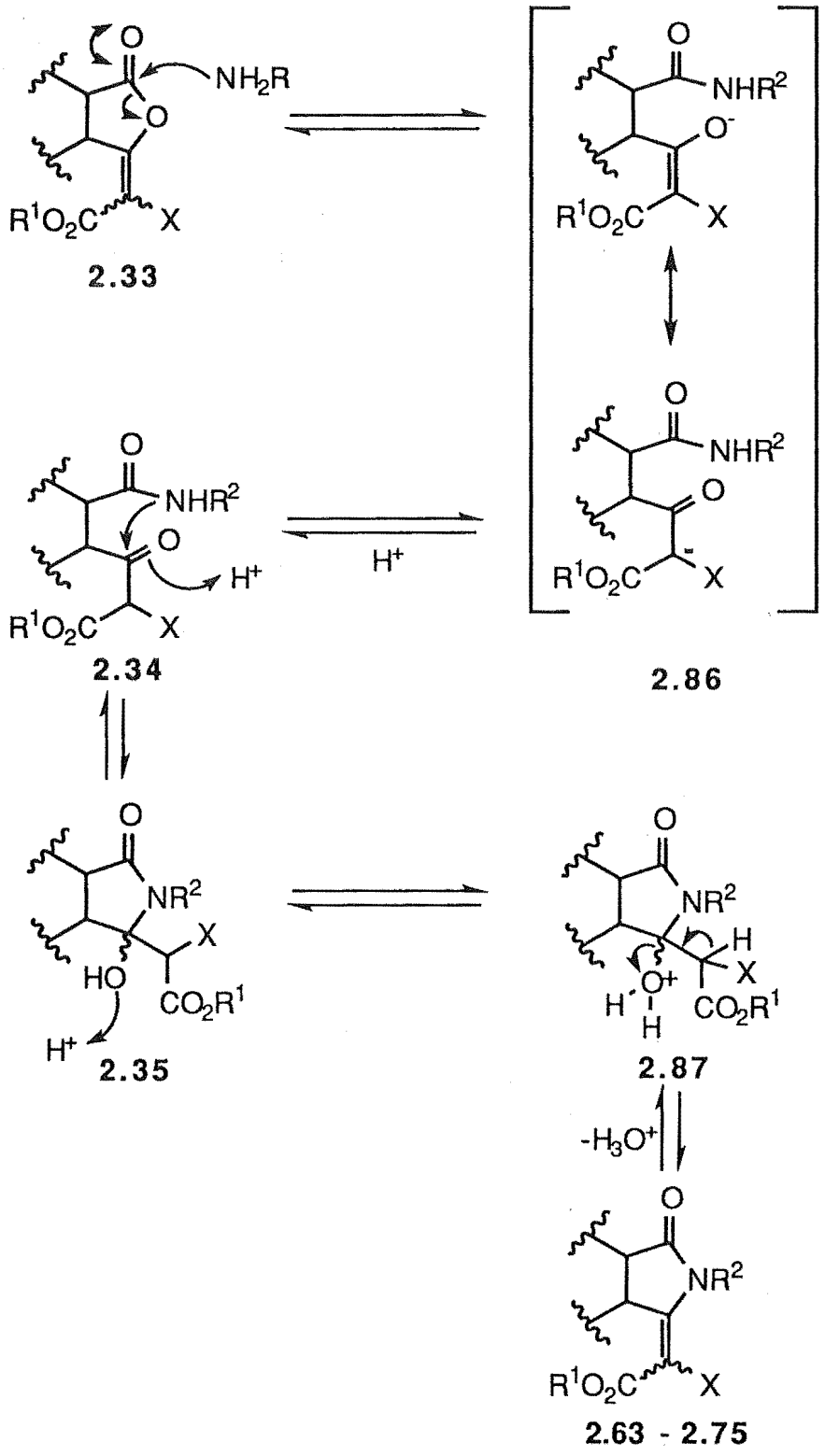


SECTION 2.5

MECHANISM OF THE INSERTION REACTION

The proposed mechanism of the insertion reaction is shown in SCHEME 2.20.

SCHEME 2.20



Initial attack at the enollactone (**2.33**) carbonyl by the amine leads, via a resonance stabilized intermediate (**2.86**), to the keto-amide (**2.34**), which was isolated in the succinimide-based series (**2.37-2.47**, TABLE 2.03, *Section 2.2.1*). The keto-amide then cyclizes to the hydroxy lactam (**2.35**), which was isolated in the phthalimide-based series and in the NH_3-derived series (**2.49-2.50**, TABLE 2.03, *Section 2.2.1*). Loss of H_2O from the hydroxy lactam (**2.35**) results in the formation of E- and Z-enamino esters (**2.63-2.75**).

Evidence for this mechanism comes from the isolation and characterization of keto-amides and hydroxy lactams (**2.37-2.47** and **2.48-2.50**, respectively, TABLE 2.03, *Section 2.2.1*) which were shown to be precursors of enamino esters. Also, the NH_3-derived keto-amide (**2.37**) formed the corresponding hydroxy lactam (**2.48**) on standing, which indicates that keto-amides are likely precursors of hydroxy lactams. Further, the proposed mechanism (SCHEME 2.20) requires protonation of the hydroxyl group for enamino ester formation which is consistent with the reaction conditions used.

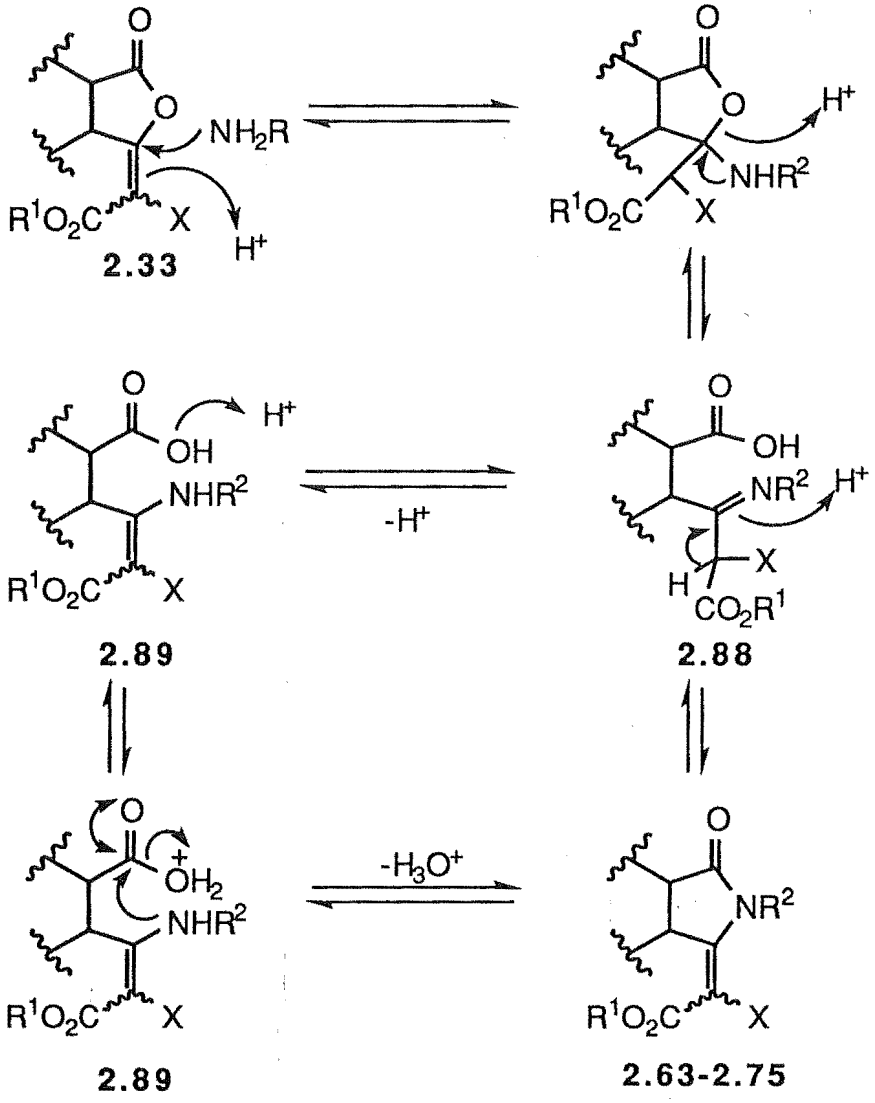
OTHER MECHANISMS

A competing mechanism (SCHEME 2.21) for the formation of enamino esters, involving Michael addition of the amine to the enollactone must also be considered. Whereas the mechanism depicted in SCHEME 2.20 leads to the isolation of keto-amide (**2.34**) and/or hydroxy lactam (**2.35**) intermediates, the mechanism depicted in SCHEME 2.21 is expected to lead to the isolation of imine (**2.88**) and/or enamine (**2.89**) intermediates. However, the spectral data of the keto-amide and hydroxy lactam intermediates (**2.37-2.47** and **2.48-2.50**, respectively, SCHEME 2.09) were inconsistent with the presence of imine (**2.88**) and/or enamine (**2.89**). For example, the ^{13}C NMR spectra did not contain signals at δ 145-160 or at δ 125-160 which are characteristic of imine[2.21] and enamine[2.22] carbons, respectively. Also, the enamine (**2.89**) and possibly also the imine (**2.88**) would be expected to exhibit E/Z isomerism and this was not observed in the ^1H or ^{13}C NMR spectra. The enamine (**2.89**) can also be discounted as an intermediate isolated during the insertion reaction on the basis of ^1H NMR integrals. An N**H** resonance was not observed in the ^1H NMR spectra of hydroxy lactams (**2.49-2.50**). When X=H, the enamine (**2.89**) contains an olefinic proton; however, the ^1H NMR spectrum of neither the

keto-amides (**2.37-2.47**) or the hydroxy lactams (**2.48-2.50**) contained a resonance attributable to an olefinic proton.

Therefore, it is highly unlikely that the mechanism depicted in SCHEME 2.21 operates in competition with the mechanism depicted in SCHEME 2.20 for the synthesis of enamino esters (**2.63-2.75**) from enollactones and amines.

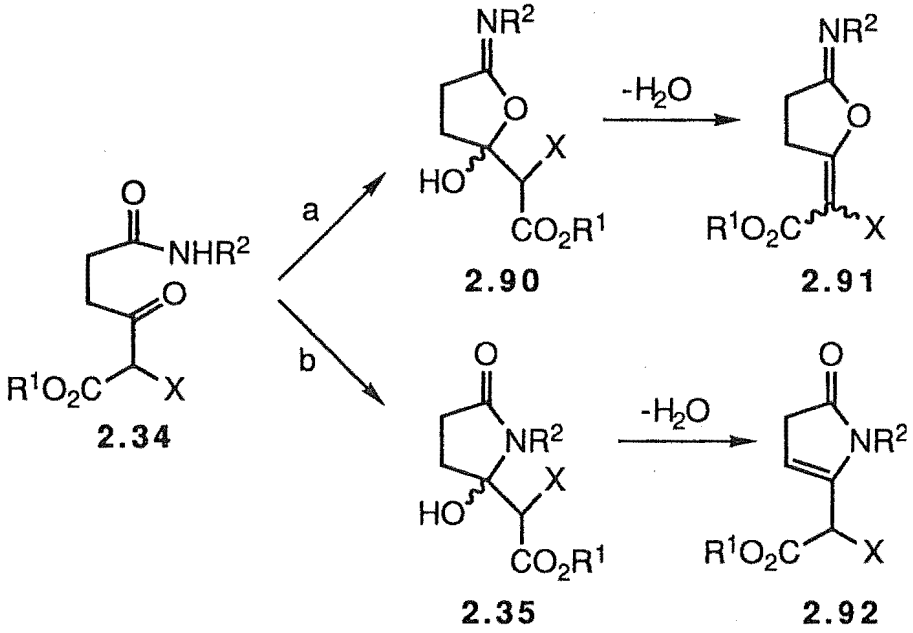
SCHEME 2.21



OTHER POSSIBLE PRODUCTS

It is conceivable that the keto-amide (**2.34**), as well as giving rise to enamino esters, might give rise to the imine (**2.91**) or the endocyclic compound (**2.92**) as shown in pathways a and b, respectively, of SCHEME 2.22. However, the crude ^{1}H and ^{13}C NMR spectra indicated that the enamino esters (**2.63-2.75**) were the sole reaction products (with the exception of the methyl enamino esters (**2.67, 2.73**) discussed earlier in *Section 2.4*). Hence it is unlikely that either of the pathways depicted in SCHEME 2.22 operates.

SCHEME 2.22



The imine (**2.91**) might be expected to exhibit E/Z isomerism at both the imine and alkene, which was not observed in the ^{1}H and ^{13}C NMR spectra of the enamino esters (**2.63-2.75**). Also, the ^{13}C NMR spectra did not have resonances characteristic of the imine carbon[2.21].

The ^{1}H NMR spectra of the methyl enamino esters (**2.67, 2.73**) did not contain resonances attributable to the olefinic and C**H**Me protons of the endocyclic isomer (**2.92**). Also, the phthalimide-based enamino esters (**2.74-2.75**) are unable to exist as endocyclic isomers.

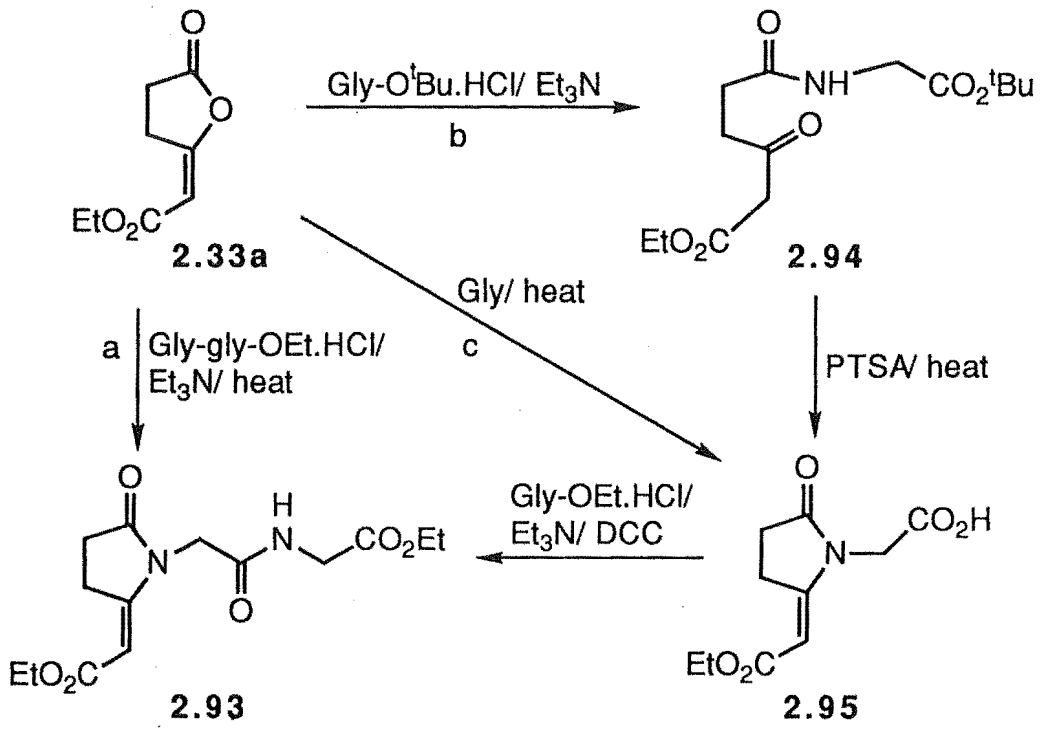
SECTION 2.6

EXTENSION OF THE PEPTIDE CHAIN IN THE C DIRECTION

The ultimate goal of this work was to develop the insertion reaction for the synthesis of peptide analogue inhibitors of serine proteases (**i.34** and **i.35**, *Section I.5*, Introduction) The insertion reaction allows for the extension of the peptide chain in the C direction. Serine protease substrate recognition is influenced by interactions between the enzyme and residues removed from the primary binding amino acid residue (S_1). Therefore, the incorporation of a potential inhibitor into a small peptide is a strategy for increasing specificity towards a target enzyme. Enamino esters (**2.68-2.73, 2.75**) extend in the C direction by one amino acid residue. C direction extension by two amino acid residues, which would provide an additional binding site, is discussed in this section.

Gly-gly enamino ester (**2.93**) was synthesized via 2 routes; directly by insertion of glycylglycine ethylester into the enollactone (**2.33a**) (pathway a, SCHEME 2.23) and also by the stepwise addition of glycine residues (pathway b, SCHEME 2.23).

SCHEME 2.23



The better method, with a yield of 79%, was the direct reaction whereby enollactone (**2.33a**), glycylglycine ethylester hydrochloride (1.8 equivalent) and triethylamine (1.8 equivalent) in 1, 2-dichloroethane were refluxed for 16h, with azeotropic removal of H_2O, to yield crude enamino ester (**2.93**) which was purified by radial chromatography. Enamino ester (**2.93**) was assigned the E configuration on the basis of the chemical shift of the (**H-4**)$_2$ resonance; δ 3.32, which indicated deshielding by CO_2Et, and the =C**H** coupling constant; J=2.0Hz (*cf Section 2.3.2*).

The stepwise addition of glycine units to the enollactone (**2.33a**) gave the enamino ester (**2.93**) in a yield of 47% (SCHEME 2.23). In the first step of this 3 step reaction, the keto-amide (**2.94**) was isolated after reaction of enollactone (**2.33a**), glycine *tert*-butylester hydrochloride (1.5 equivalent) and triethylamine (1.5 equivalent), at 20 °C. The keto-amide (**2.94**) was refluxed for 5h, with azeotropic removal of H_2O, in 1, 2-dichloroethane containing PTSA, to yield the deprotected enamino ester (**2.95**). From the chemical shift of the (**H-4**)$_2$ protons; δ 3.30 and the coupling constant of =C**H**; J=1.8Hz, enamino ester (**2.95**) was assigned the E configuration (*cf Section 2.3.2*). Other enamino esters (**2.63-2.75**) prepared via this method were washed with H_2O to remove the PTSA (*Section 2.3.1*); however, enamino ester (**2.95**) was significantly soluble in H_2O, hence it was used without purification. Thus, enamino ester (**2.95**) was dissolved in CH_2Cl_2, treated with DCC (1,3-dicyclohexylcarbodiimide), glycine ethylester hydrochloride (1.1 equivalent) and triethylamine (1.1 equivalent) and the resulting mixture was stirred at 20 °C for 16h. Work-up gave the crude enamino ester (**2.93**) contaminated with some DCC and DCC by-products. Purification by radial chromatography yielded pure E-enamino ester (**2.93**).

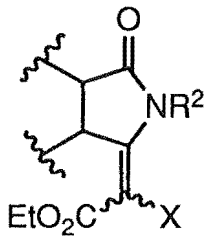
Enamino ester (**2.95**) was also prepared directly via the reaction of enollactone (**2.33a**) with glycine (SCHEME 2.23, pathway c). However, due to the insolubility of glycine in organic solvents, it was necessary to perform the reaction in CH_2Cl_2 (5mL)/DMF (5mL)/H_2O (3mL). After stirring at 20 °C for 44h, the solution was heated at reflux temperature and the solvent was allowed to evaporate. The residue contained, by ^1H NMR spectroscopy, E-enamino ester (**2.95**) contaminated with DMF. As expected, the

corresponding keto-amide precursor was not isolated. This illustrates that the insertion reaction may be performed with amino acids as well as esters of amino acids.

SECTION 2.7

(ATTEMPTED) SYNTHESES OF HALO ENAMINO ESTERS

Enamino esters (**i.34**) which are potential mechanism-based inactivators of serine proteases, contain a latent reactive group; an α-halo imine (*Section I.5*, Introduction). This section examines attempts to prepare enamino esters (**2.36**) with potential latent reactivity.



2.36 X = Br, Cl

SECTION 2.7.1

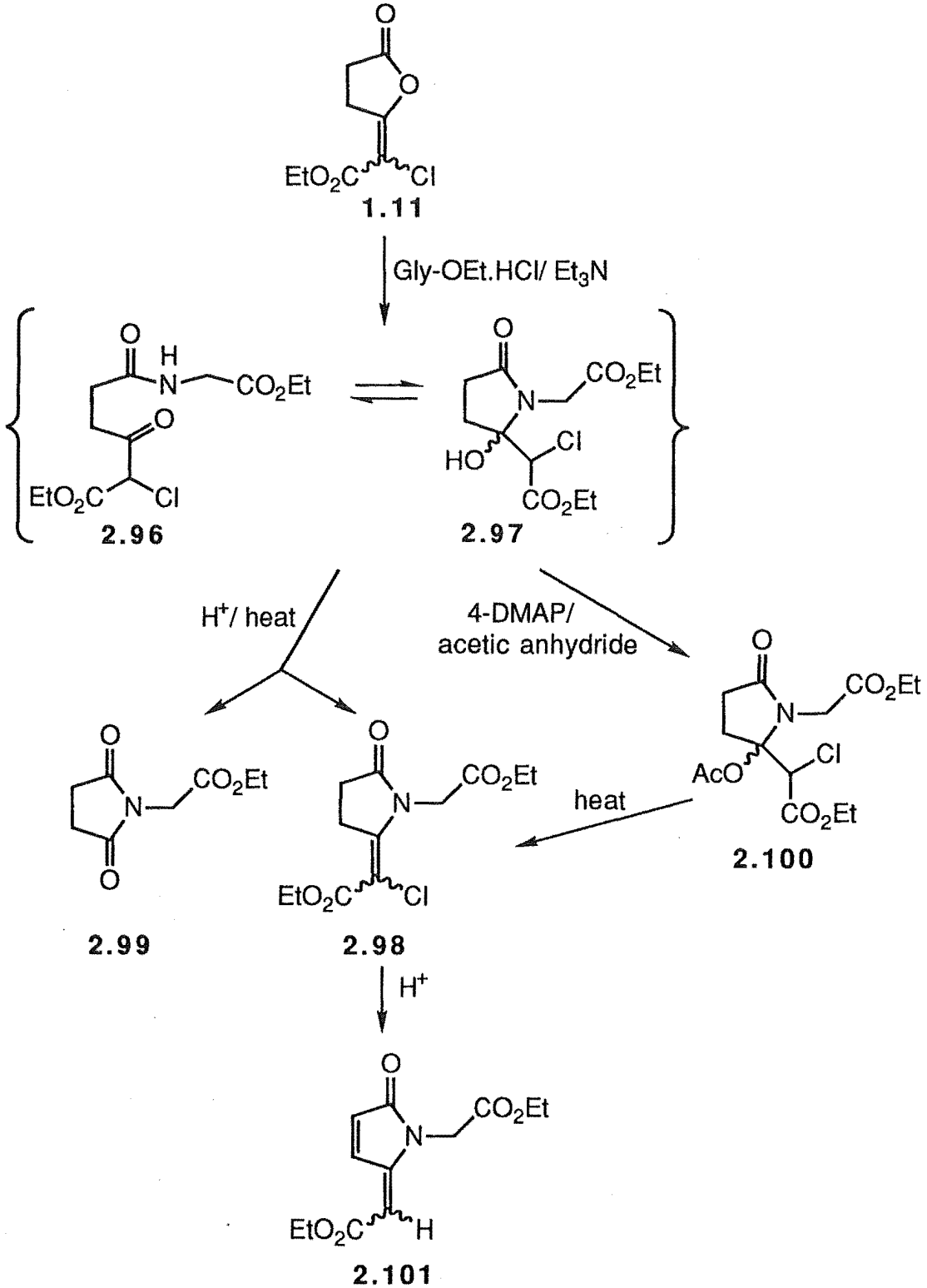
SYNTHESIS OF SUCCINIMIDE-BASED CHLORO ENAMINO ESTERS

SCHEME 2.24 summarizes the reactions used to prepare enamino ester (**2.98**). Chloro enollactone (**1.11**), glycine ethylester hydrochloride (1.3 equivalent) and triethylamine (1.3 equivalent) were stirred for 3h in ethyl acetate. The solvent was evaporated under reduced pressure and a ^{1}H NMR spectrum of the residue indicated that keto-amide (**2.96**) and hydroxy lactam (**2.97**) were present in a ratio of 9 : 1, respectively. Ethyl acetate was added to the residue, the mixture was filtered to remove triethylamine hydrochloride and the solvent was evaporated under reduced pressure to yield a mixture of keto-amide (**2.96**) and hydroxy lactam (**2.97**) in a ratio of 3 : 1, respectively, by ^{1}H NMR spectroscopy.

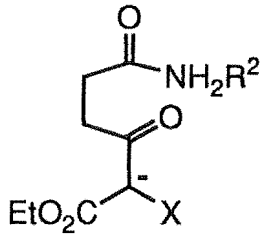
The keto-amides (**2.37-2.47**, TABLE 2.03) were normally isolated rather than the corresponding hydroxy lactams in the succinimide-based series (*Section 2.2.1*).

Isolation of hydroxy lactam (**2.97**) may be attributable to the electronegative chlorine making the ketone carbon of the keto-amide (**2.96**) more electrophilic and therefore more susceptible to reaction with the amide nitrogen and/or to the "gem-dimethyl effect" (also known as the "Thorpe-Ingold effect")[2,23].

SCHEME 2.24



The chloro keto-amide (**2.96**) formed more readily than the corresponding protio and methyl keto-amides (**2.37-2.47**, TABLE 2.03, *Section 2.2.1*). The more electronegative chlorine is likely to stabilize the negative charge of the keto-amide precursor (**2.86**), thus making formation of keto-amide (**2.96**) more facile.



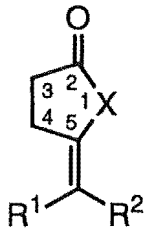
2.86 X = Cl

The hydroxy lactam (**2.97**), which contains 2 chiral centres, was found, by ^{1}H NMR spectroscopy, to be present as a mixture of 2 diastereoisomers. Due to overlapping signals it was not possible to estimate the ratio of diastereoisomers.

Dehydration of (**2.96/2.97**) was effected via heating with PTSA in 1, 2-dichloroethane, containing activated 4Å sieves, at 70 °C for 6.5 days. The mixture was filtered and purification by radial chromatography yielded E- and Z-chloro enamino esters (**2.98**) in a 1 : 1 ratio by ^{1}H NMR spectroscopy. The Z isomer (**2.98Z**) was assigned on the basis of a downfield shift of 0.35ppm for $(\mathbf{H\text{-}4})_2$ relative to the E isomer (**2.98E**). The assignment was supported by the ^{1}H NMR spectrum of the E- and Z-chloro enollactones (**1.11**) in which $(\mathbf{H\text{-}4})_2$ resonated at a similar chemical shift to $(\mathbf{H\text{-}4})_2$ in the E- and Z-enamino ester (**2.98**), respectively (TABLE 2.07).

In the ^{13}C NMR spectrum of the chloro enamino esters, the C-5 resonance was downfield in the Z isomer (**2.98Z**) relative to the E isomer (**2.98E**). This trend was also observed in the ^{13}C NMR spectra of chloro and bromo enollactones (*Section 1.8*, Chapter 1) and is analogous to the trend observed for protio and methyl enamino esters (**2.67**, **2.73**, **2.74-2.75**, *Section 2.3.2*).

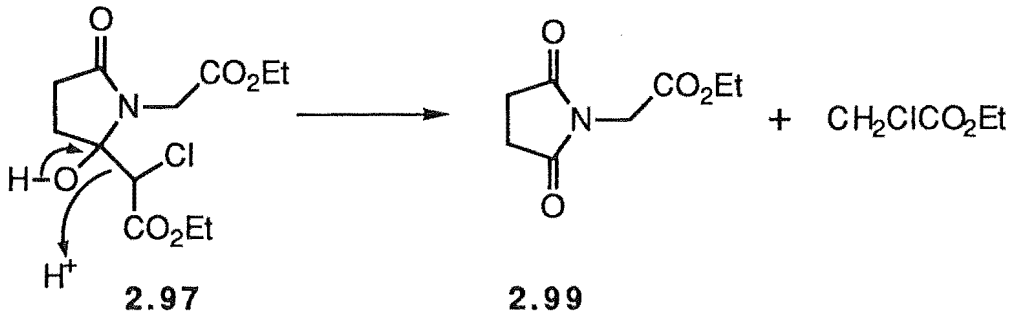
TABLE 2.07: δ **(H-4)**$_2$ and δ **C-5** in E-and Z-Chloro Enamino Esters and Enollactones



Compd. & Confign.	R^1	R^2	X	δ **(H-4)**$_2$	δ **C-5**
2.98 E	Cl	CO_2Et	NCH_2CO_2Et	3.00	150.2
2.98 Z	CO_2Et	Cl	NCH_2CO_2Et	3.35	152.3
1.11 E	Cl	CO_2Et	O	3.14	158.5
1.11 Z	CO_2Et	Cl	O	3.43	161.5

The yield of E- and Z-enamino esters (**2.98**) was very low; 26%, due to a competing pathway, involving a retro-Claisen type reaction leading to formation of the imide (**2.99**, SCHEME 2.24 and SCHEME 2.25). The melting point and spectral data of the imide (**2.99**) were identical to the melting point and spectral data previously reported[2.24] for the imide (**2.99**).

SCHEME 2.25



The retro-Claisen type reaction was not observed for any of the previously discussed methyl or protio keto-amides or hydroxy lactams (**2.63-2.75**, *Section 2.2.1* and **2.94**, *Section 2.6*). Again, chlorine is better able to stabilize the negative charge of the leaving group, thus making imide (**2.99**) formation more favourable for the chloro hydroxy lactam (**2.97**) (SCHEME 2.25).

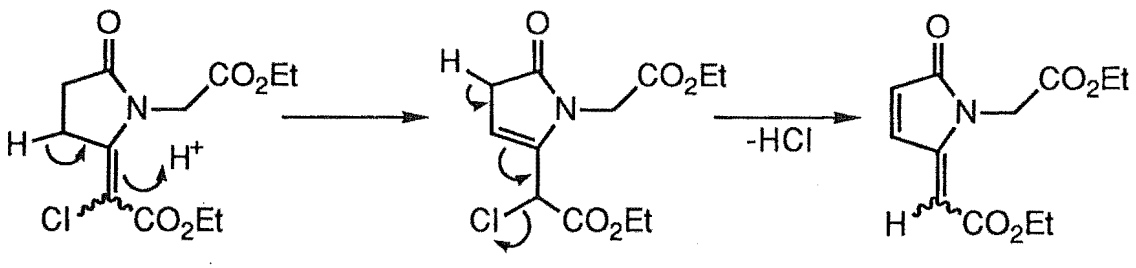
Imide (**2.99**) formation was effectively blocked by first making the acetate (**2.100**) of the hydroxy lactam (**2.97**) (SCHEME 2.24). This was achieved by stirring (**2.96/2.97**), acetic anhydride (2 equivalent), triethylamine (2 equivalent) and 4-dimethyl amino pyridine (4-DMAP) (1.5 equivalent) in CH_2Cl_2 for 2.5h. ^1H NMR spectroscopy indicated the acetate (**2.100**) was present as a mixture of two diastereoisomers, in a ratio of 3 : 2. The acetate (**2.100**) was heated at 65 °C in benzene for 90min to eliminate acetic acid. Evaporation of the solvent under reduced pressure yielded E- and Z-enamino esters (**2.98**); 44% E : 56% Z by ^1H NMR spectroscopy, in a much improved yield of 78%.

However, the E- and Z-chloro enamino esters (**2.98**) were unstable. On heating and/or in the presence of acid, E- and Z-chloro enamino esters (**2.98**) eliminated HCl to give the E and Z isomers of (**2.101**) (SCHEME 2.24). For example, attempted distillation of the enamino esters (**2.98**), formed via the acetate route, led to complete conversion to the elimination product (**2.101**). The elimination product (**2.101**) also formed from chloro enamino esters (**2.98**) during radial chromatography on silica.

The elimination product (**2.101**) was prepared by refluxing a 1, 2-dichloroethane solution of (**2.96/2.97**) and PTSA for 3h with azeotropic removal of H_2O. The yield of elimination product (**2.101**) after purification by radial chromatography was relatively low; 39%, due to the competing retro-Claisen type reaction to imide (**2.99**).

The proposed mechanism for formation of the elimination product (**2.101**), shown in SCHEME 2.26, is similar to that leading to formation of the methyl endocyclic isomers (**2.80-2.81**, SCHEME 2.19, *Section 2.4*); however, for the chloro enamino esters (**2.98**) subsequent elimination of HCl was possible.

SCHEME 2.26



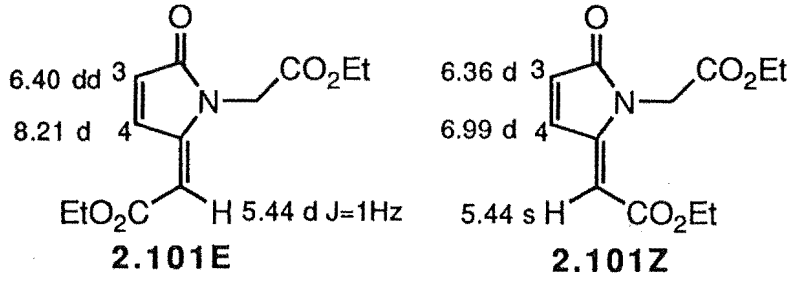
2.98 **2.102** **2.101**

Heating a 1 : 1 $CDCl_3$ solution of E- and Z-chloro enamino esters (**2.98**), at 55 °C, showed that the E-enamino ester (**2.98E**) formed the elimination product (**2.101**) more rapidly than the Z-isomer (**2.98Z**). After 15min all of the E isomer (**2.98E**) had been converted to the elimination product (**2.101**), whereas it took 45min for the Z-enamino ester (**2.98Z**) to be completely converted to elimination product (**2.101**). The reason for the more rapid reaction of E-enamino ester (**2.98E**) to elimination product (**2.101**) is unknown.

The structure of the elimination product (**2.101**) was established from the spectral data. The mass spectrum was consistent with the absence of Cl and the accurate mass was consistent with the expected mass of elimination product (**2.101**). The ^1H and ^{13}C NMR spectra indicated that the basic enamino ester structure of (**2.98**) was intact and that E and Z isomers were present. Major changes involved the introduction of three olefinic protons and the positions of these in the structure was determined by the respective chemical shifts and the results of ^1H NMR decoupling experiments.

Assignment of configuration to the isomers of the elimination product (**2.101**) was based upon ^1H NMR spectroscopy (SCHEME 2.27) (*cf Section 2.3.2*). The E isomer (**2.101E**) showed the characteristic downfield shift of **H-4** relative to the Z isomer (**2.101Z**) due to the deshielding effect of CO_2Et. The Z isomer (**2.101Z**) showed a downfield shift for NCH_2 relative to the E isomer (**2.101E**), also reflecting the deshielding effect of CO_2Et. Further, as expected, the olefinic proton, $=CHCO_2Et$; of the E isomer (**2.101E**) exhibited a greater degree of coupling to **H-3** than the corresponding olefinic proton of the Z isomer (**2.101Z**).

SCHEME 2.27

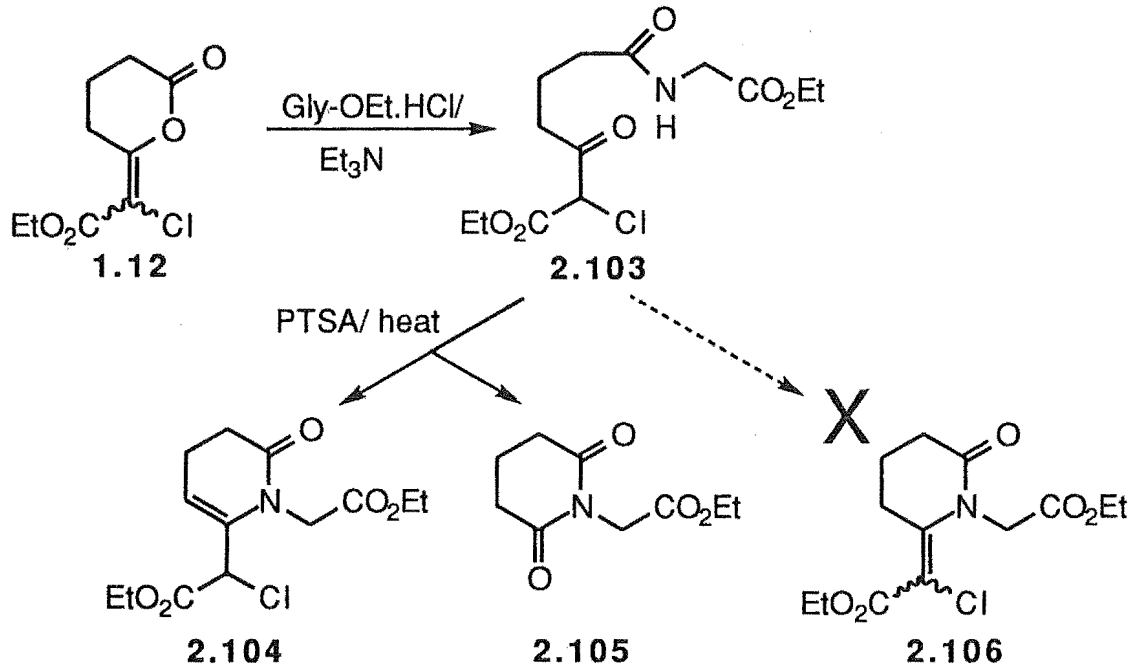


SECTION 2.7.2

ATTEMPTED SYNTHESIS OF GLUTARIMIDE-BASED CHLORO ENAMINO ESTERS

The keto-amide (**2.103**) was isolated, in 84% yield, from the reaction of chloro enollactone (**1.12**) with glycine ethylester hydrochloride (1.4 equivalent) and triethylamine (1.4 equivalent) at 20 °C (SCHEME 2.28).

SCHEME 2.28



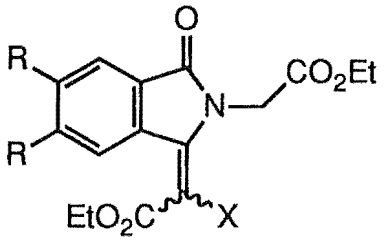
The keto-amide (**2.103**) was refluxed in 1, 2-dichloroethane containing PTSA for 6h, with azeotropic removal of H_2O, to give the endocyclic isomer (**2.104**). Radial chromatography gave a fraction containing the endocyclic isomer (**2.104**) (38%) and another fraction containing a mixture of unreacted keto-amide (**2.103**) (10%) and imide (**2.105**) (8%). Imide (**2.105**) was formed by the competing retro-Claisen type reaction, which was also observed in the chloro succinic series (SCHEME 2.24, *Section 2.7.1*). The presence of the imide (**2.105**), a known compound[2.24], was implied in the ^1H NMR spectrum by a triplet at δ 2.53 arising from 2x $COCH_2$ and a singlet at δ 4.51 arising from NCH_2.

The ^{1}H NMR spectrum of the main product was consistent with endocyclic enamino ester (**2.104**) rather than the corresponding exocyclic enamino ester (**2.106**); a singlet at δ 4.97 indicated the CHCl resonance, a triplet at δ 5.62 indicated the =C**H** resonance, and the introduction of a chiral centre was reflected by the presence of diastereotopic protons. It was not surprising that the endocyclic enamino ester (**2.104**) formed in preference to the corresponding exocyclic enamino ester (**2.106**) because six-membered rings are known to be thermodynamically more stable with an endocyclic, rather than an exocyclic, double bond[2.25].

SECTION 2.7.3

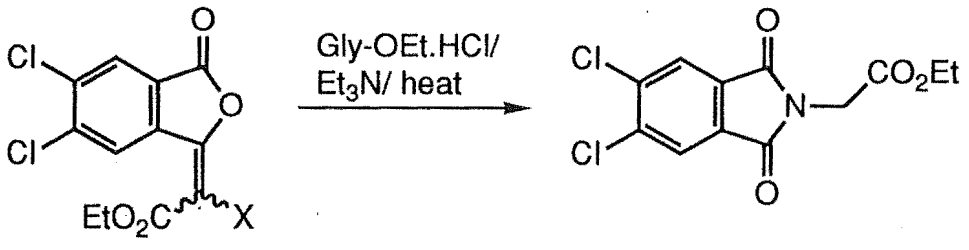
ATTEMPTED SYNTHESES OF PHTHALIMIDE-BASED HALO ENAMINO ESTERS

Attempts at forming phthalimide-based halo enamino esters (**2.107-2.109**), via reaction of phthalimide-based halo enollactones (**1.17**, **1.15** and **1.14**, respectively) with glycine ethylester were unsuccessful. Imides (**2.110**, SCHEME 2.29 and **2.111**, pathway a SCHEME 2.30, respectively), which presumably arose from the retro-Claisen type reaction discussed earlier (*Section 2.7.1*), were the only identified products.



	X	R
2.107	Br	Cl
2.108	Cl	Cl
2.109	Cl	H

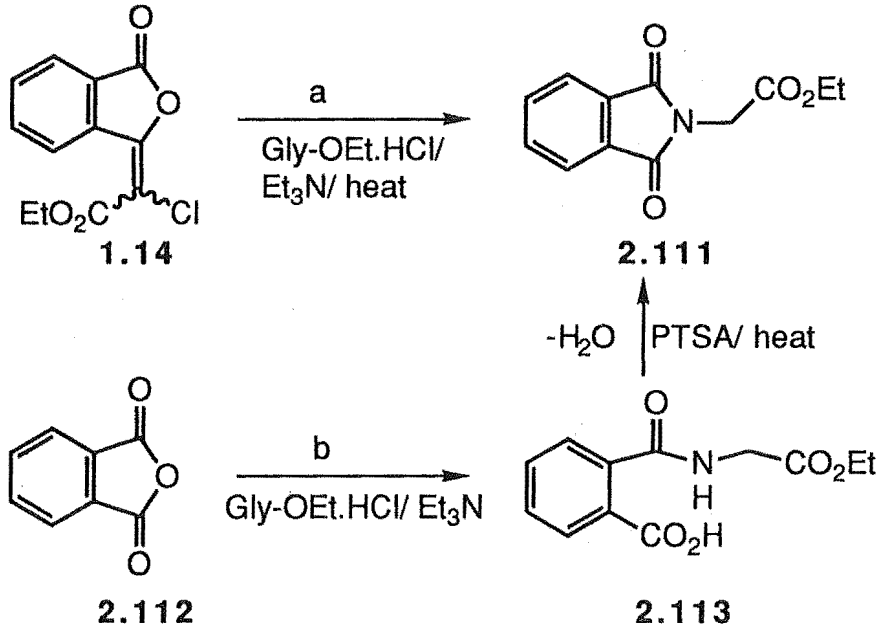
SCHEME 2.29



1.15 X = Cl
1.17 X = Br

2.110

SCHEME 2.30



Imide formation occurred more readily than for the chloro succinic and glutaric series (*Sections 2.7.1* and *2.7.2*). The succinimide- and glutarimide-based imides (**2.99** and **2.105**, respectively) formed when the keto-amide (and hydroxy lactam) (**2.96/2.97**, **2.103**) were heated, whereas the phthalimide-based imides (**2.110** and **2.111**) were observed after the 20 °C reaction of phthalic enollactones (**1.15**, **1.17** and **1.21**) with glycine ethylester hydrochloride (1.3-1.4 equivalent) and triethylamine (1.3-1.4 equivalent) in CH_2Cl_2 or ethyl acetate. The relative amounts of imides (**2.110** and **2.111**) increased on heating the mixtures in 1, 2-dichloroethane with PTSA. After radial chromatography of the reaction mixture arising from bromo enollactone (**1.17**), imide (**2.110**) was obtained in a yield of 49%. The imide (**2.110**) was identified by comparison of its spectral data with that of the known[2.24] imide (**2.111**). No other fractions contained compounds which were identified.

Imides (**2.110** and **2.111**), present in the reaction mixtures arising from enollactones (**1.15** and **1.21**, respectively), were not isolated. However, imide (**2.111**) was prepared independently, in an overall yield of 48%, via dehydration of the keto-amide (**2.113**) formed from the reaction of phthalic anhydride (**2.112**) with glycine ethylester (pathway b, SCHEME 2.30). Dehydration of the keto-amide (**2.113**) required refluxing with PTSA in 1, 2-

dichloroethane, with azeotropic removal of H_2O, for 8 days. The imide (**2.111**) was identified by comparison of its melting point and spectral data with literature values[2.24].

Further attempts were made to obtain bromo enamino ester (**2.107**) from the corresponding bromo enollactone (**1.17**) using the method that successfully blocked imide formation in the succinimide-based series (*Section 2.7.1*). Thus a mixture containing enollactone (**1.17**), glycine ethylester hydrochloride (1.3 equivalent) and triethylamine (1.3 equivalent), in ethyl acetate, was stirred at 20 °C for 50min. Acetic anhydride (2 equivalent), triethylamine (2 equivalent) and 4-DMAP (1.5 equivalent) were added and the resulting mixture was stirred for 2h. The ^1H NMR spectrum of the residue, obtained after work-up, contained the imide (**2.110**) and other unidentified compounds.

Three separate portions of the residue were heated under different conditions; at 70 °C in C_6D_6 for 1 day, at reflux in toluene for 16h, and at 180 °C at 1mm for 2h. The only species identified by ^1H NMR spectroscopy, even after purification of the latter two residues by radial chromatography, was the imide (**2.110**).

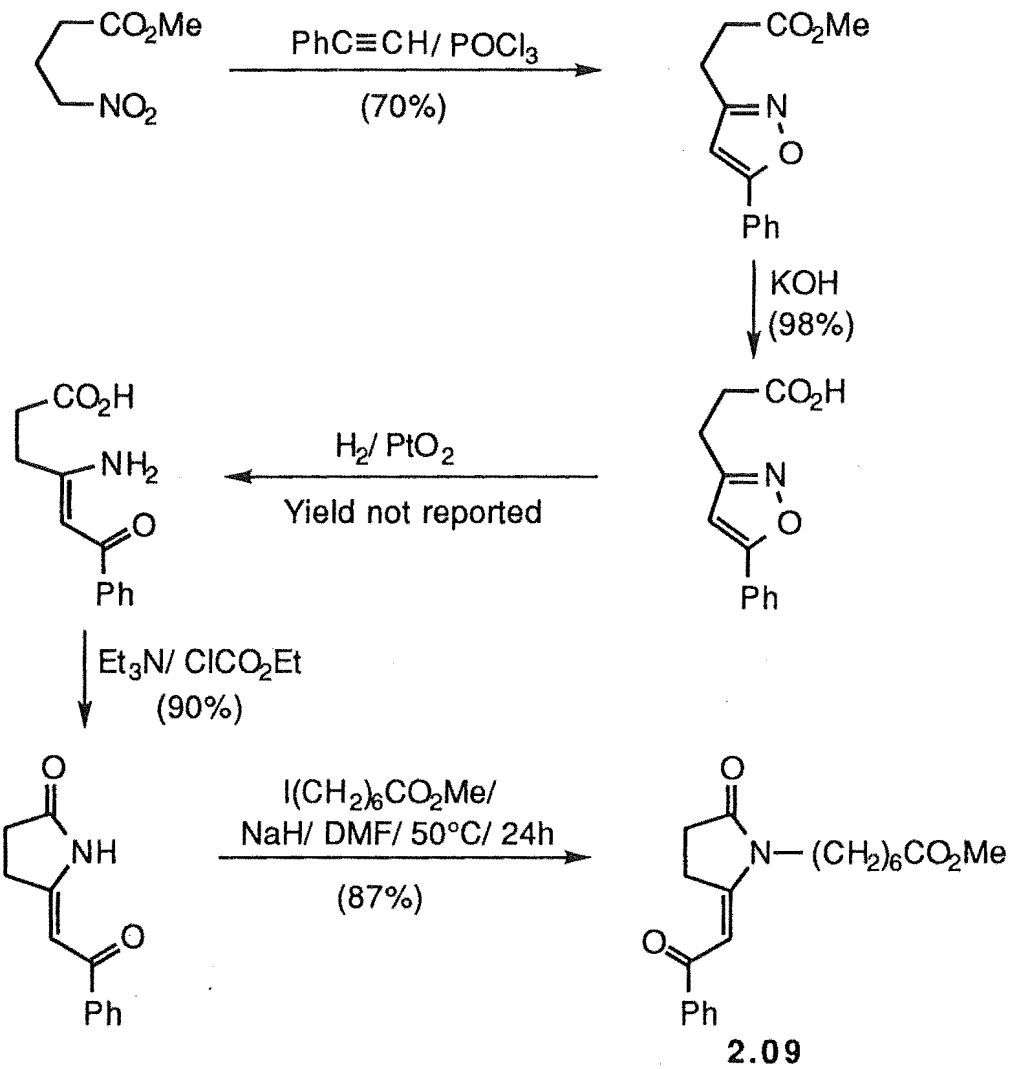
Therefore, phthalimide-based halo enamino esters (**2.107-2.109**) were not able to be synthesized via the insertion reaction. Instead, the reactions led to formation of the imides (**2.110-2.111**) and several unidentified compounds. Imides are potentially useful compounds with respect to enzyme inhibition[2.26].

SECTION 2.8

SYNTHESIS OF A KEY SYNTHETIC INTERMEDIATE OF PROSTAGLANDIN ANALOGUES USING THE INSERTION REACTION

As mentioned at the beginning of this chapter, enamino esters are a sub-class of the group of compounds known as ene-lactams. The ene-lactam (**2.09**) is a key synthetic intermediate of prostaglandin analogues (TABLE 2.01, *Section 2.1*). Ene-lactam (**2.09**) has been prepared, in a yield of 54%, via the 5 step sequence shown in SCHEME 2.31[2.05]. (Since no yield was reported for the reduction reaction, the overall yield may have been less than 54%.)

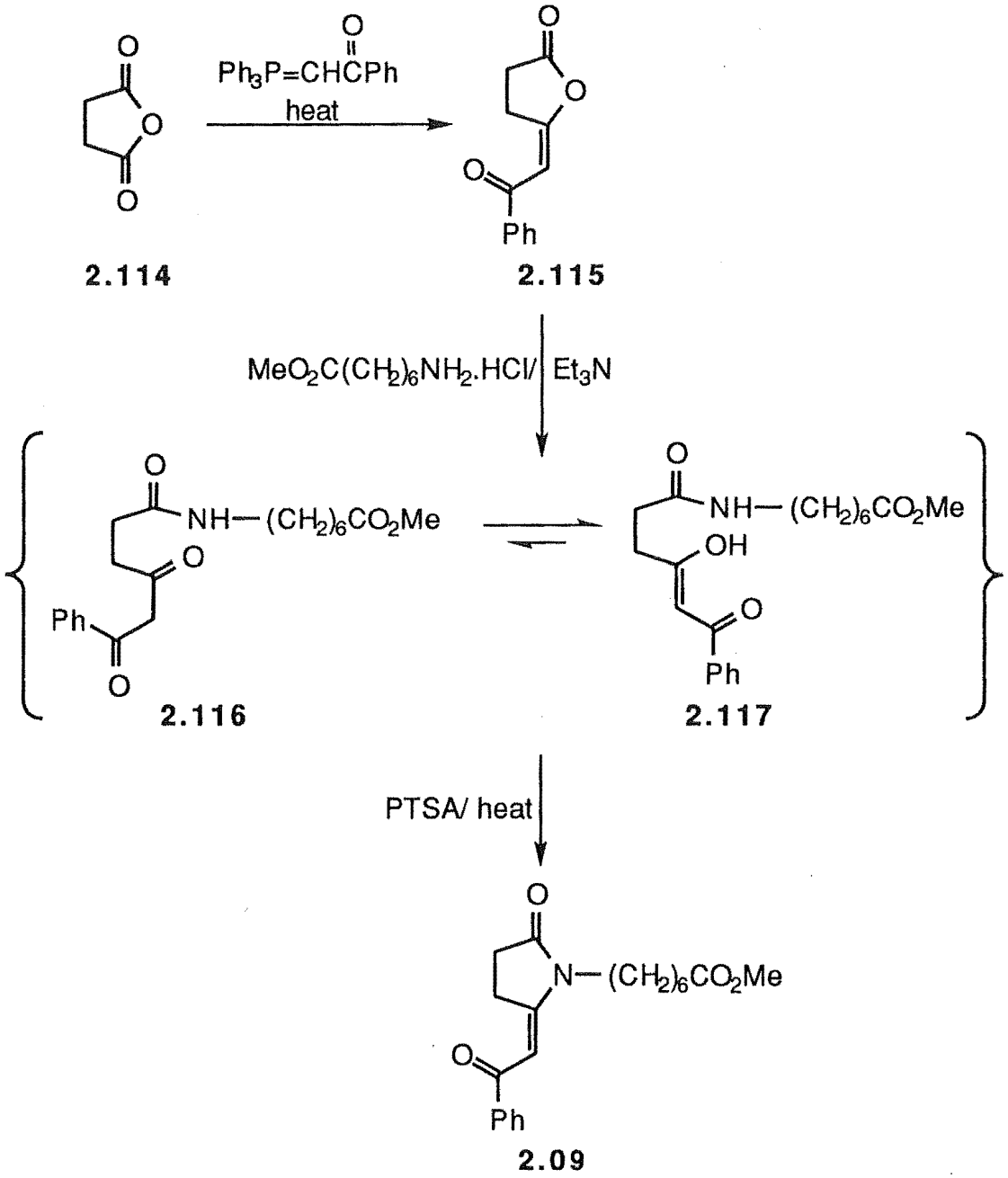
SCHEME 2.31



2.09

Using the Insertion reaction ene-lactam (**2.09**) was synthesized, in the improved yield of 75%, via the 3 step sequence outlined in SCHEME 2.32.

SCHEME 2.32



The appropriate enollactone (**2.115**) was prepared in a yield of 99% via standard Wittig chemistry. However, it was necessary to reflux the CH_2Cl_2 solution of succinic anhydride (**2.114**) and Ph_3P=CHCOPh (2.3 equivalent) for a total of 5 months. The reaction

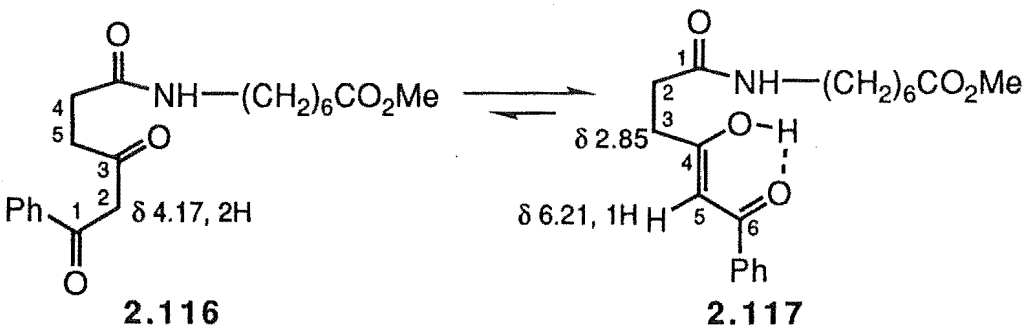
was not accelerated by the higher boiling point solvents toluene and $CHCl_3$. The enollactone (**2.115**), which was purified by column chromatography, was assigned the E configuration on the basis of the chemical shift of (**H-4**)$_2$; δ 3.64 and the =CH coupling constant of J=2.1Hz (*cf Section 2.3.2*).

Reaction of enollactone (**2.115**) with heptanoic methylester hydrochloride (1.3 equivalent) and triethylamine (1.3 equivalent) in CH_2Cl_2 gave, in a yield of 93%, a mixture of the keto-amide (**2.116**) and the corresponding enol-amide (**2.117**), in a ratio of 1:4, respectively, by ^1H NMR spectroscopy.

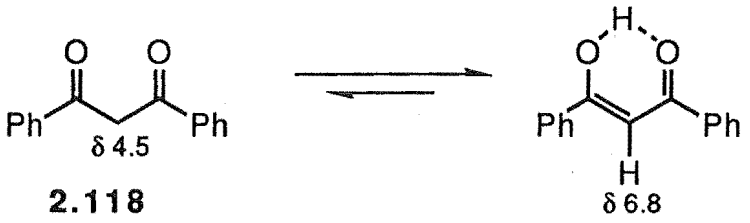
The enol form (**2.117**) predominated over the keto form (**2.116**), due to stabilization by double bond conjugation with the phenyl group. The keto-amides (**2.37-2.47**, TABLE 2.03, *Section 2.2.1*) do not have the phenyl substituent and existed entirely in the keto form (*Section 2.2.1*).

It is likely that the geometry about the enol double bond is Z due to the potential for intramolecular hydrogen bond formation between the hydroxyl and the carbonyl (SCHEME 2.33).

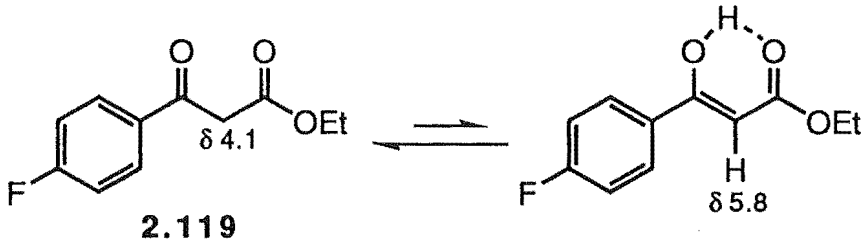
SCHEME 2.33



The chemical shift of (**H-3**)$_2$; δ 2.85, which does not indicate deshielding by COPh, supports the assignment of the Z configuration to the enol amide (**2.117**). The chemical shift of =C**H** (δ 6.21) for the enol-amide (**2.117**) and (**H-2**)$_2$ (δ 4.17) for the keto-amide (**2.116**) compares well with analogous chemical shifts reported for the keto and enol forms of (**2.118** and **2.119**)[2.26].



2.118



2.119

The IR spectrum also provided evidence for the enol-amide (**2.117**); a carbonyl stretch, observed at 1720-1730cm^{-1} for other keto-amides (**2.38-2.39**, **2.44-2.45**, **2.47**, Table 2.03, *Section 2.2.1*) was absent from the IR spectrum of (**2.117**).

The keto-amide/enol-amide mixture (**2.116/2.117**) in 1, 2-dichloroethane containing PTSA, was refluxed, with azeotropic removal of H_2O, for 43h to give the ene-lactam (**2.09**) in a yield of 81%. Ene-lactam (**2.09**) was assigned the E configuration on the basis of the chemical shift of the (**H-4**)$_2$ resonance; δ 3.44, and the =CH coupling constant of J=1.8Hz (*cf Section 2.3.2*).

Ene-lactam (**2.09**) synthesized via the alternative literature route (SCHEME 2.31) was depicted[2.05] as having the Z configuration. This is unlikely because acylated enamino esters (for example **2.64-2.75**) have been found to exist in the E configuration (*Section 2.3.2*). Also, the method for the synthesis of the ene-lactam (**2.09**, SCHEME 2.31) required heating at 50 °C for 24h; conditions known to promote the conversion of Z isomers to E isomers (*Section 2.3*). The melting point and spectral data for ene-lactam (**2.09**) synthesized via the routes outlined in SCHEMES 2.31 and 2.32 were identical. Thus, the insertion reaction was applied, with better results, to the synthesis of an ene-lactam which represents a key synthetic intermediate of prostaglandin analogues[2.05] (**2.13**, TABLE 2.01, *Section 2.1*).

SECTION 2.9

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CHAPTER 3

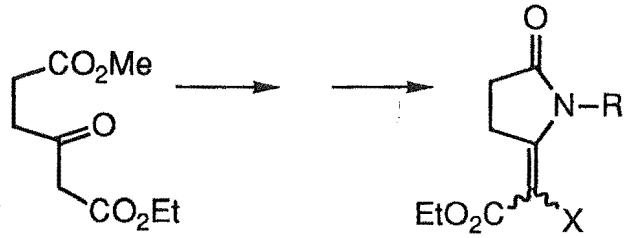
SYNTHESIS OF ENAMINO ESTERS FROM β-KETO ESTERS

SECTION 3.1

INTRODUCTION

Protio and bromo cyclic acylated enamino esters are potential alternate substrate and mechanism-based inhibitors, respectively, of serine proteases (*Section 1.5*, Introduction). This chapter describes the synthesis of protio and bromo cyclic acylated enamino esters (**2.66**, **2.71**, **3.02-3.03**, SCHEME 3.01) from the reaction of β-keto ester (**3.01**) with glycine ethyl ester hydrochloride or butylamine, via isolable enamine intermediates. Protio enamino esters (**2.66** and **2.71**) have been prepared via the insertion reaction (*Sections 2.2-2.3*, Chapter 2).

SCHEME 3.01

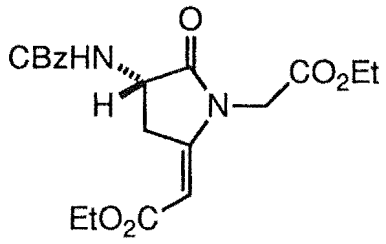


3.01

	X	R
2.66	H	^{n}Bu
2.71	H	CH_2CO_2Et
3.02	Br	^{n}Bu
3.03	Br	CH_2CO_2Et

A related procedure has been reported[3.01] in which β-keto ester (**2.29**) is refluxed with various amines in toluene for 18h. The mixture is then treated with NaH to yield the E-enamino esters (**2.30**) (SCHEME 2.06, *Section 2.1*, Chapter 2). In this case, an intermediate species was not isolated.

Also in this chapter, the β-keto ester route and insertion reaction are extended to the synthesis of the 3-substituted enamino ester (**3.04**), which has the potential for peptide chain extension in the N and C directions. Incorporation of enamino esters into an appropriate oligopeptide is a strategy for enhancing recognition by a target enzyme.



3.04

SECTION 3.2

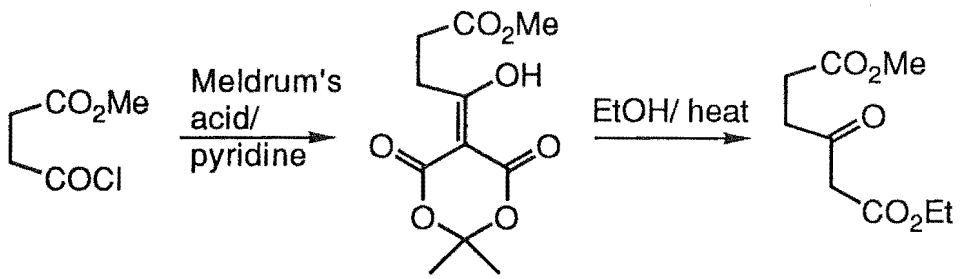
SYNTHESIS OF PROTIO AND BROMO ENAMINO ESTERS

SECTION 3.2.1

PREPARATION OF β-KETO ESTER

β-Keto ester (**3.01**), the starting compound for the synthesis of enamino esters (**2.66, 2.71, 3.02, 3.03**, SCHEME 3.01), was prepared from acid chloride[3.02] (**3.05**) according to the procedure reported by Oikawa et al[3.03] (SCHEME 3.02). A CH_2Cl_2 solution of acid chloride (1.1 equivalent) and pyridine (2 equivalent) was treated with Meldrum's acid (2,2-dimethyl-1,3-dioxane-4,6-dione) at 0 °C for 1h and then at 20 °C for 1h, to give the acyl Meldrum's acid (**3.06**). Acyl Meldrum's acid (**3.06**) was refluxed in ethanol for 2h to give β-keto ester (**3.01**) which was used subsequently without purification.

SCHEME 3.02



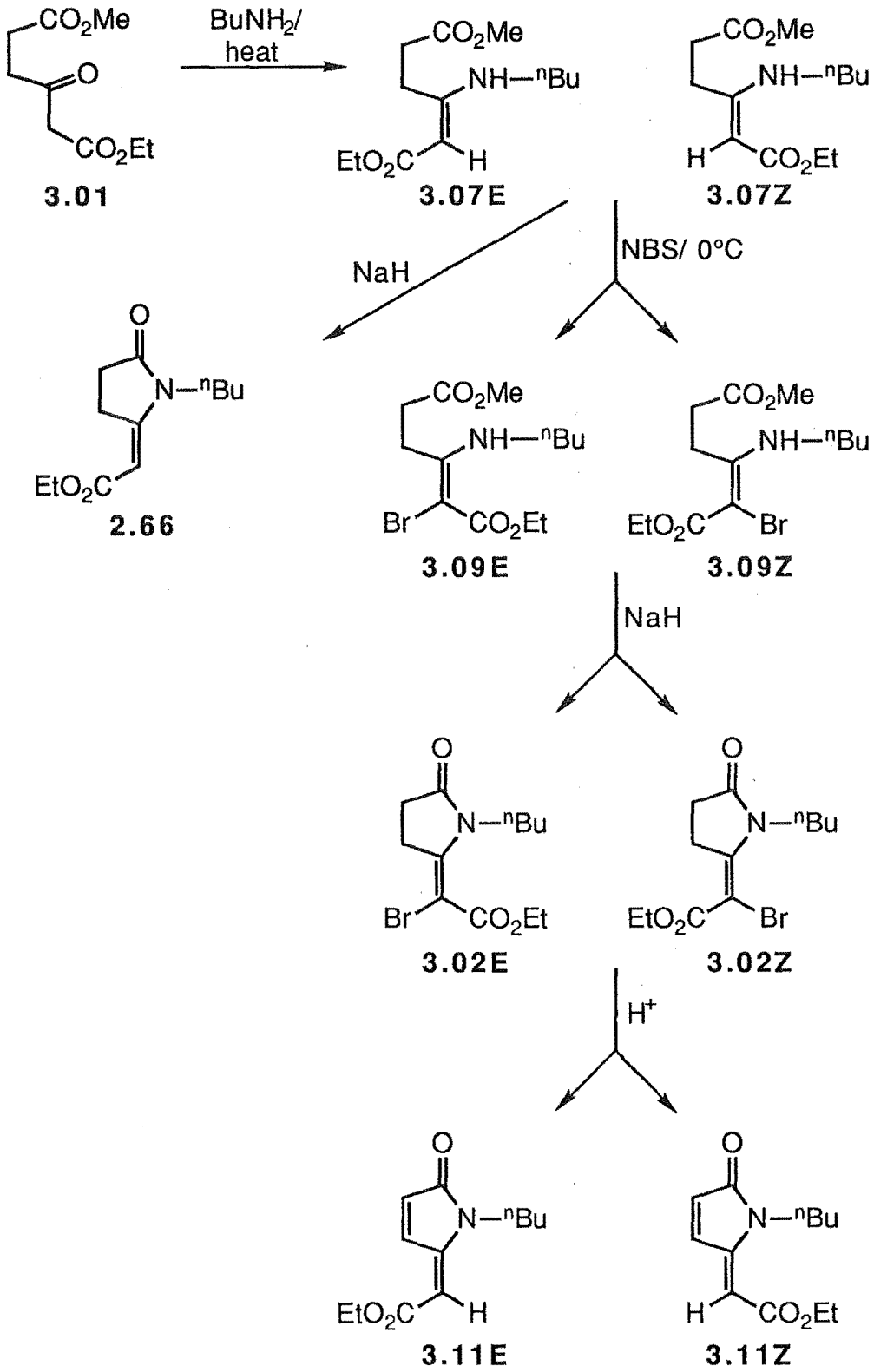
3.05 **3.06** **3.01**

SECTION 3.2.2

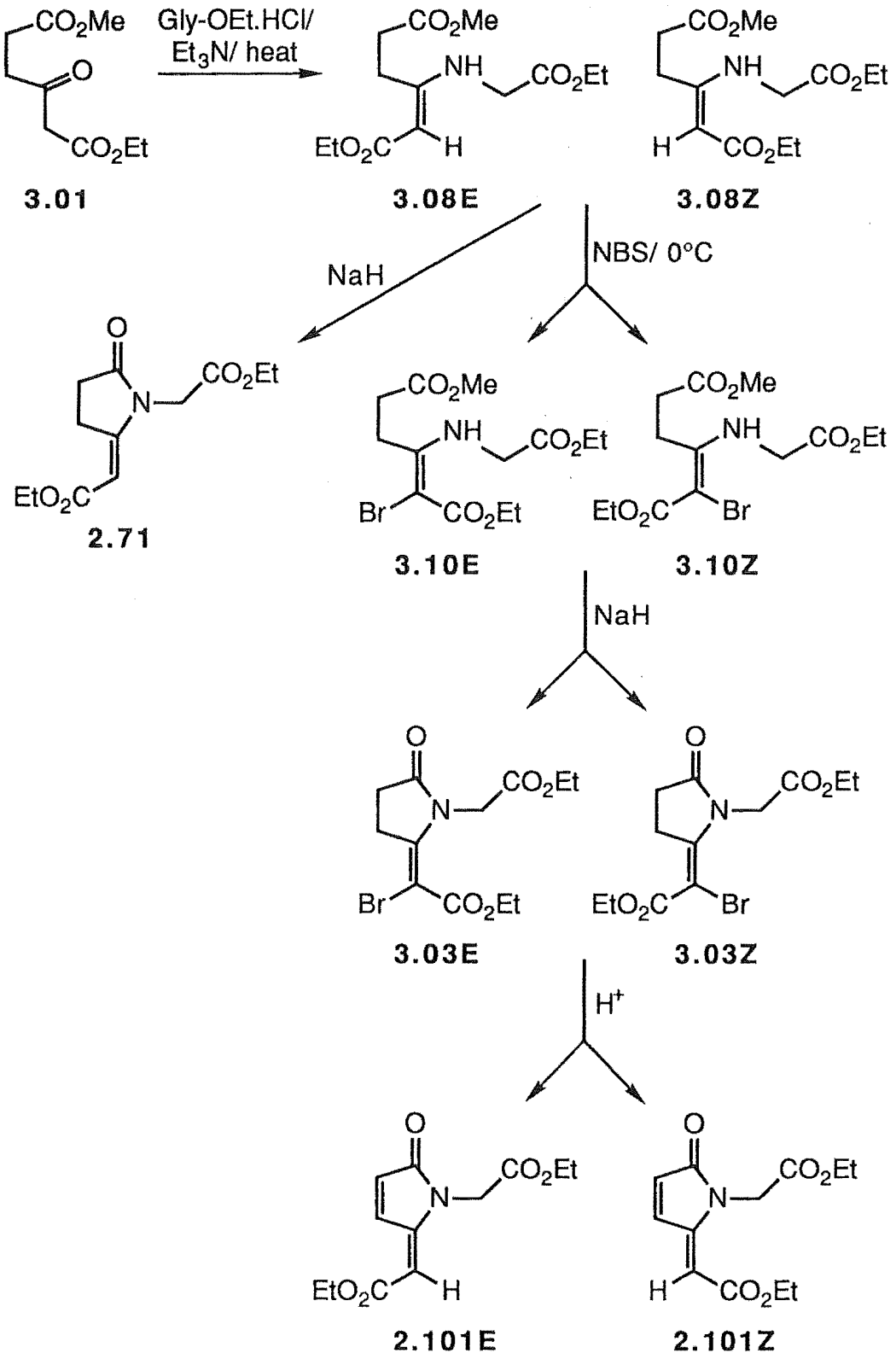
PREPARATION AND CHARACTERIZATION OF ENAMINES

Protio enamines (**3.07** and **3.08**, SCHEMES 3.03 and 3.04, respectively) were prepared by refluxing, with azeotropic removal of H_2O, a benzene solution of the β-keto ester (**3.01**) with butylamine (2 equivalent), or glycine ethylester hydrochloride (2 equivalent) and triethylamine (2 equivalent). After 90min the solvent was evaporated and the crude enamines (**3.07** and **3.08**) were purified by radial chromatography.

SCHEME 3.03


CO2Me
O
CO2Et
3.01
BuNH2/
heat
CO2Me
NH—nBu
EtO2C
H
3.07E
CO2Me
NH—nBu
H
CO2Et
3.07Z
NaH
NBS/ 0°C
O
N—nBu
EtO2C
2.66
CO2Me
NH—nBu
Br
CO2Et
3.09E
CO2Me
NH—nBu
EtO2C
Br
3.09Z
NaH
O
N—nBu
Br
CO2Et
3.02E
O
N—nBu
EtO2C
Br
3.02Z
H+
O
N—nBu
EtO2C
H
3.11E
O
N—nBu
H
CO2Et
3.11Z

SCHEME 3.04


CO2Me
O
CO2Et
Gly-OEt.HCl/
Et3N/ heat
NH
CO2Et
EtO2C
H
3.01
3.08E
3.08Z
NaH
NBS/ 0°C
N
2.71
Br
3.10E
3.10Z
3.03E
3.03Z
H+
2.101E
2.101Z

Bromo enamines (**3.09** and **3.10**, SCHEMES 3.03 and 3.04, respectively) were prepared from the corresponding protio enamines (**3.07** and **3.08**, respectively), in 100% and 96% yield, respectively, by treatment with NBS (*N*-bromosuccinimide) (1 equivalent) at 0 °C for 15min in THF.

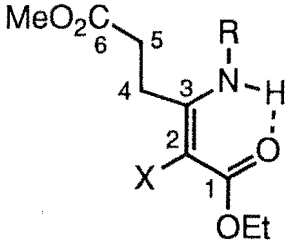
The ^{1}H NMR spectra of enamines (**3.07-3.10**) indicated that E and Z isomers were present. The broad N**H** resonances at δ 4.72, 5.31, 5.51 and 6.07 were attributed to the minor isomer of enamines (**3.07**, **3.08**, **3.09** and **3.10**, respectively). The integrals of the N**H** resonances allowed estimation of the E/Z ratio for enamines (**3.07-3.10**) (TABLE 3.01). Doubling of other resonances in the ^{1}H and ^{13}C NMR spectra of enamines (**3.07-3.10**) also indicated the presence of the minor isomer.

The chemical shift of the N**H** resonance in the major isomer of enamines (**3.07-3.10**) was indicative of hydrogen-bonding between NH and CO_2Et (SCHEME 3.05 and TABLE 3.01). Therefore, the major isomer was assigned the Z configuration for the protio enamines (**3.07** and **3.08**), and the E configuration for bromo enamines (**3.09** and **3.10**), on the basis of the downfield position of the N**H** resonance relative to the corresponding minor isomer (TABLE 3.01).

TABLE 3.01

Enamine	R	X	δ N**H**	(Confign)	δ N**H**	(Confign)	E/Z
3.07	^{n}Bu	H	8.58	(Z)	4.72	(E)	22/78
3.08	CH_2CO_2Et	H	8.89	(Z)	5.31	(E)	28/72
3.09	^{n}Bu	Br	9.28	(E)	5.51	(Z)	83/17
3.10	CH_2CO_2Et	Br	9.59	(E)	6.07	(Z)	75/25

SCHEME 3.05



3.07 Z
3.08 Z
3.09 E
3.10 E

The spectral data of (**3.07-3.10**) were consistent with the proposed enamine structure. Resonances at δ 4.44 and 4.55 in the ^{1}H NMR spectrum of Z-protio enamines (**3.07Z** and **3.08Z**, respectively) were characteristic of the olefinic enamine proton. Medium to strong absorptions at 1610-1675cm^{-1} in the IR spectra of compounds (**3.07-3.10**) are characteristic of enamines[3.04]. The olefinic enamine carbon, **C-1**, of the major isomer (**3.07Z**, **3.08Z**, **3.09E** and **3.10E**) resonated at δ 80.5, 83.5, 76.9 and 79.5, respectively, which is within the range typically observed for **C-1** of enamines; δ 79-131[3.04]. The resonances observed for **C-2** of the major isomer (**3.07Z**, **3.08Z**, **3.09E** and **3.10E**); δ >160, were downfield of the resonances typically observed for **C-2**; δ 124-156[3.04]. However, the accurate masses of enamines (**3.07-3.10**) were consistent with the calculated masses.

SECTION 3.2.3

SYNTHESIS AND CHARACTERIZATION OF ENAMINO ESTERS

Enamino esters (**2.66**, **3.02**, SCHEME 3.03 and **2.71**, **3.03**, SCHEME 3.04) were prepared from the appropriate enamine (**3.07**, **3.09**, **3.08** and **3.10**, respectively) on treatment with NaH (1 equivalent) at 20 °C for 18h, in THF.

Protio enamino esters (**2.66** and **2.71**) were identical, by ^{1}H NMR spectroscopy, to enamino esters (**2.66** and **2.71**) prepared via the insertion reaction (*Sections 2.2* and *2.3*, Chapter 2). Exclusively the E isomer formed; in yields of 40% and 27%, respectively, from the β-keto ester (**3.01**). Enamino esters (**2.66** and **2.71**) were synthesized in superior yields from enollactones, via the insertion reaction; 100% and 73%, respectively (*Sections 2.2* and *2.3*, Chapter 2). Another disadvantage of the β-keto ester route is that β-keto esters are less convenient to prepare than enollactones.

The structure of bromo enamino esters (**3.02** and **3.03**) was confirmed by comparison of their spectral data with that of related enamino esters; in particular the chloro enamino esters (**2.98**) synthesized via the insertion reaction (*Section 2.7.1*, Chapter 2). The configuration of the bromo enamino esters (**3.02** and **3.03**) was assigned on the basis of ^{1}H NMR spectroscopy. The (**H-4**)$_2$ resonance was downfield in the Z isomers

(**3.02Z** and **3.03Z**) relative to the corresponding E isomers (**3.02E** and **3.03E**, respectively), reflecting the deshielding effect of CO_2Et.

ELIMINATION PRODUCT

Bromo enamino esters (**3.02** and **3.03**, SCHEMES 3.03 and 3.04, respectively), like the chloro enamino ester (**2.98**, *Section 2.7.1*, Chapter 2), were unstable. The residue obtained from the reaction of the glycine-derived bromo enamine (**3.10**) with NaH, contained, by ^1H NMR spectroscopy, 85% bromo enamino ester (**3.03E** and **3.03Z**) (in a ratio of 1 E : 4 Z) and 15% elimination product (**2.101E** and **2.101Z**) (in a ratio of 3 E : 2 Z). When this residue was subjected to preparative tlc on silica, the E- and Z-bromo enamino esters (**3.03E** and **3.03Z**, respectively) were separated. However, the fraction containing the E isomer (**3.03E**) was contaminated with elimination product (**2.101E** and **2.101Z**); 30% (**3.08E**) and 70% (**2.101E** and **2.101Z**), by ^1H NMR spectroscopy. The elimination product (**2.101E** and **2.101Z**) was also obtained from the corresponding chloro enamino ester (**2.98**) (*Section 2.7.1*, Chapter 2). This reaction may represent a convenient preparation of this class of compound.

The residue obtained from the reaction of the butylamine-derived bromo enamine (**3.09**) with NaH, contained, by ^1H NMR spectroscopy, 30% bromo enamino ester (**3.02E** and **3.02Z**) (in a ratio of 7 E : 3 Z) and 70% elimination product (**3.11E** and **3.11Z**) (in a ratio of 7 E : 3 Z). Complete conversion to elimination product (**3.11E** and **3.11Z**) was achieved when the residue was dissolved in CCl_4 containing *p*-toluene sulphonic acid (PTSA) and heated at 60 °C for 5 days. Purification by radial chromatography yielded elimination product (**3.11E** and **3.11Z**, respectively) in a ratio of 9 E : 1 Z, by ^1H NMR spectroscopy. The E isomer of the elimination product (**3.11E**) was assigned on the basis of the downfield shift of **H-4** and the larger coupling constant of =CH relative to the Z isomer (**3.11Z**).

A reduced reaction time of 2h, rather than 18h, for the butylamine-derived bromo enamine (**3.09**)/NaH reaction gave, by ^1H NMR spectroscopy, 80% bromo enamino ester (**3.02E** and **3.02Z**) (in a ratio of 7.5 E : 2.5 Z) and 20% elimination product (**3.11E** and **3.11Z**) (in a ratio of 7.5 E : 2.5 Z). Purification by radial chromatography allowed separation

of E- and Z-bromo enamino esters (**3.02E** and **3.02Z**, respectively); however, the fraction containing the E isomer (**3.026E**) was also contaminated with elimination product (**3.11E** and **3.11Z**); 40% (**3.02**) : 60% (**3.11**), by ^{1}H NMR spectroscopy.

SECTION 3.3

SYNTHESIS OF AN ENAMINO ESTER WITH THE POTENTIAL FOR PEPTIDE CHAIN EXTENSION IN THE N DIRECTION

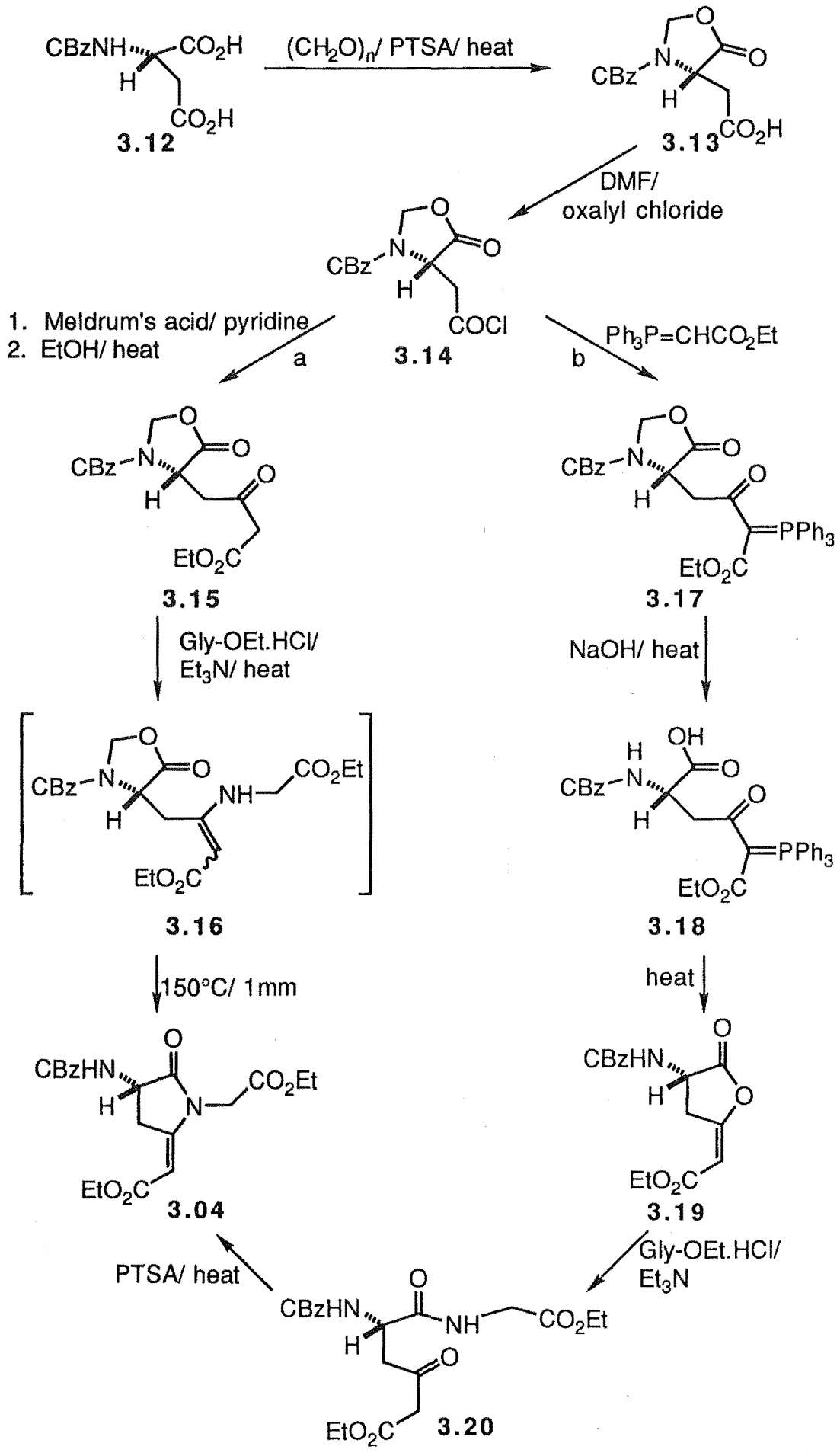
Enamino ester (**3.04**) (SCHEME 3.06), which has the potential for peptide chain extension in the N and C directions, was synthesized via the enamine route (pathway a, SCHEME 3.06) and the insertion reaction (pathway b, SCHEME 3.06). The starting compound for both syntheses was the acid chloride (**3.14**), which was prepared from the reaction of the corresponding acid (**3.13**) with oxalyl chloride (10 equivalent) and DMF in CH_2Cl_2. The acid (**3.13**) was synthesized according to the procedure reported by Scholtz and Bartlett[3.05] whereby (S)-benzyloxycarbonyl (CBz) aspartic acid (**3.12**), paraformaldehyde (2 equivalent) and PTSA (0.06 equivalent), in benzene, were refluxed for 1h with azeotropic removal of H_2O. The oxazolidinone (**3.13**) effectively protects the non R group acid of (S)-CBz aspartic acid (**3.12**) and allows subsequent reactions to occur at the unprotected acid.

SECTION 3.3.1

SYNTHESIS OF ENAMINO ESTER (**3.04**) FROM β-KETO ESTER

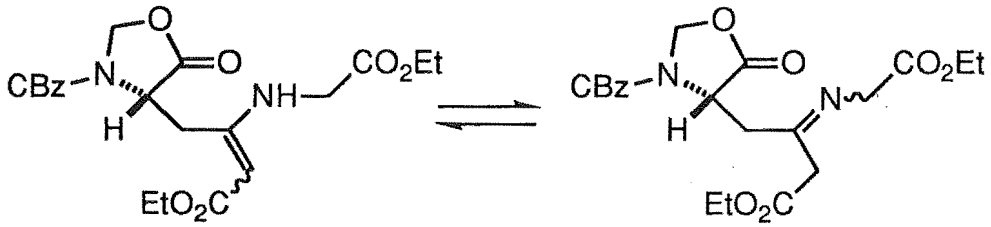
β-Keto ester (**3.15**) was prepared from the acid chloride (**3.14**), in a yield of 75%, using the procedure[3.03] used to prepare β-keto ester (**3.01**, SCHEME 3.02, *Section 3.2.1*). Thus, acid chloride (**3.14**) (1.1 equivalent), pyridine (2 equivalent) and Meldrum's acid, dissolved in CH_2Cl_2, were stirred at 0 °C for 1h then 20 °C for 1h (SCHEME 3.06). The solvent was evaporated under reduced pressure and the residue was refluxed with ethanol for 2h to give the crude β-keto ester (**3.15**) which was purified by radial chromatography.

SCHEME 3.06


CBzNH
CO2H
H
CO2H
3.12
(CH2O)n/ PTSA/ heat
CBz
N
O
O
H
CO2H
3.13
DMF/
oxalyl chloride
COCl
3.14
1. Meldrum's acid/ pyridine
2. EtOH/ heat
a
b
Ph3P=CHCO2Et
EtO2C
3.15
PPh3
3.17
Gly-OEt.HCl/
Et3N/ heat
NaOH/ heat
NH
CO2Et
3.16
OH
H
3.18
150°C/ 1mm
heat
CBzHN
3.04
3.19
PTSA/ heat
Gly-OEt.HCl/
Et3N
3.20

β-Keto ester (**3.15**), glycine ethylester hydrochloride (1.5 equivalent) and triethylamine (1.5 equivalent) were refluxed in benzene for 90min, with azeotropic removal of H_2O. The mixture was cooled to 20 °C, filtered and evaporation of the solvent under reduced pressure yielded a residue which was used without further purification. By analogy to the simpler systems (*Section 3.2.2*) the compound present at this stage ought to be the enamine (**3.16**). However, the ^1H NMR spectrum of the residue was complex and was not able to be assigned, which suggested that a mixture of E- and Z-enamines (**3.16**) and possibly also the corresponding E- and Z-imines (**3.21**) had formed (SCHEME 3.07).

SCHEME 3.07

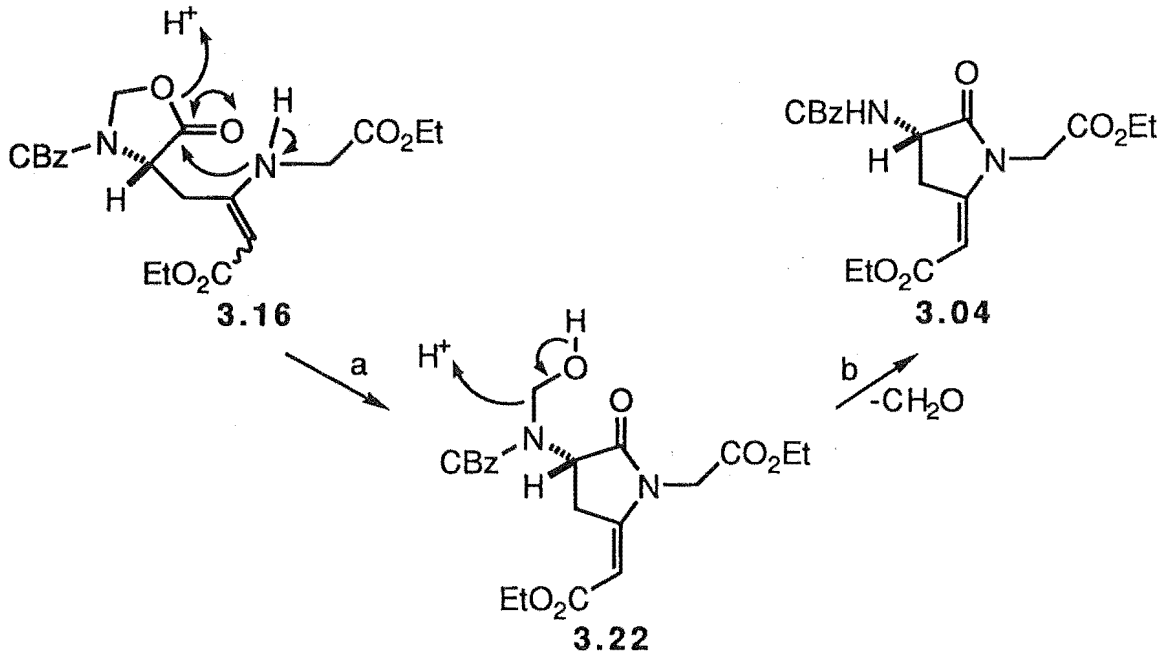


3.16 **3.21**

It is unlikely that the residue was predominantly the product of an undesirable side reaction because, on heating at 150 °C at 1mm Hg for 2h, the target enamino ester (**3.04** SCHEME 3.06) was formed relatively cleanly. Purification by radial chromatography yielded the enamino ester (**3.04**) in an overall yield of 42% from the acid chloride (**3.14**).

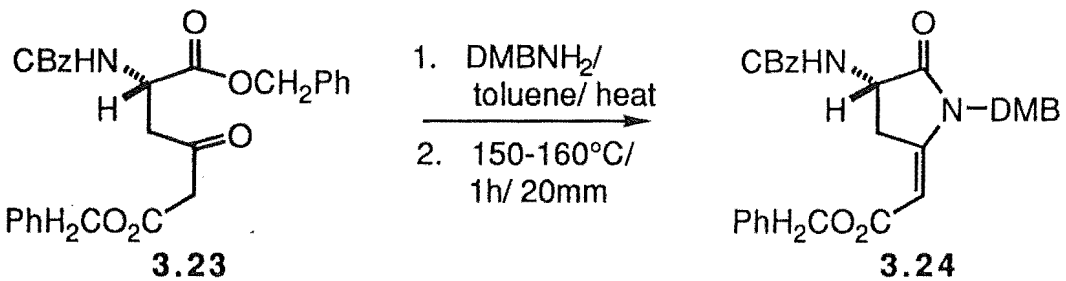
Mechanistically, the formation of enamino ester (**3.04**) from the enamine (**3.16**), on heating, involves 2 steps; formation of the 5-membered enamino ester ring via attack of the enamine N on the oxazolidinone carbonyl (step a, SCHEME 3.08) and loss of the formaldehyde protecting group (step b, SCHEME 3.08).

SCHEME 3.08



For the simpler systems (*Section 3.2.3*) the enamino esters (**2.66**, **3.02**, SCHEME 3.03 and **2.71**, **3.03**, SCHEME 3.04) were prepared from the corresponding enamines (**3.07**, **3.09** and **3.08**, **3.10**, respectively) via reaction with NaH, because this method was used for the preparation of related enamino esters[3.01]. Enamino ester (**3.04**); however, was prepared from the enamine/imine (**3.16/3.21**) via heating under reduced pressure; conditions similar to those reported[3.06] for the preparation of the related 3-substituted enamino ester (**3.24**) from β-keto ester (**3.23**) (SCHEME 3.09). The enamino esters (**2.66**, **3.02**, SCHEME 3.03 and **2.71**, **3.03**, SCHEME 3.04) were not isolated following heating, at reduced pressure, of the enamines (**3.07**, **3.09** and **3.08**, **3.10**, respectively).

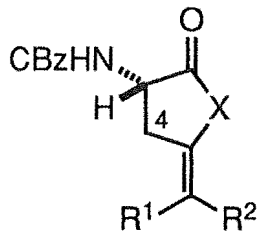
SCHEME 3.09



DMB = 2,4-dimethoxybenzyl

The ^{1}H and ^{13}C NMR spectra of the enamino ester (**3.04**) indicated a single isomer which was assigned the E configuration because the $(\mathbf{H\text{-}4})_2$ resonances were in a characteristic downfield position reflecting the deshielding effect of the CO_2Et group (*cf Section 2.3.2, Chapter 2*). The chemical shifts of $(\mathbf{H\text{-}4})_2$ were similar to the chemical shifts of $(\mathbf{H\text{-}4})_2$ in related enollactones and enamino esters (**3.24E**, **3.25E**, **3.26E**, **3.27Z**) of the same relative configuration (TABLE 3.02).

TABLE 3.02



Compd	R^1	R^2	X	δ $(\mathbf{H\text{-}4})_a$	δ $(\mathbf{H\text{-}4})_b$
	Ester group (R^1) is trans relative to X				
3.04E	CO_2Et	H	NCH_2CO_2Et	3.04	3.77
3.24E	CO_2CH_2Ph	H	NCH_2DMB	2.80-3.21	3.96-4.43
3.25E	CO_2CH_2Ph	H	NH	3.04	3.77
3.26E	CO_2Et	H	O	3.25	3.88
3.27Z	CO_2Et	Br	O	3.27	3.86
	Ester group (R^2) is cis relative to X				
3.25Z	H	CO_2CH_2Ph	NH	2.80	3.26
3.27E	Br	CO_2Et	O	3.08	3.48

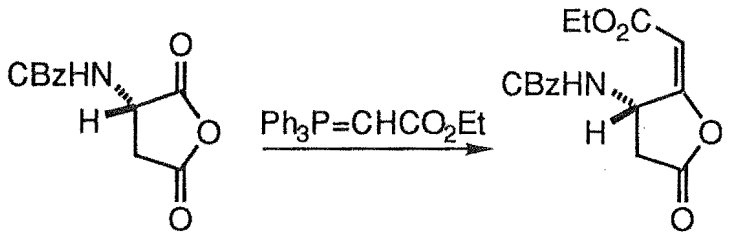
DMB = 2,4-dimethoxybenzyl

SECTION 3.3.2

SYNTHESIS OF ENAMINO ESTER (**3.04**) VIA THE INSERTION REACTION

Enamino ester (**3.04**) was also prepared via the insertion reaction (pathway b, SCHEME 3.06), which is discussed in Chapter 2. Synthesis of the enollactone (**3.19**) via anhydride Wittig chemistry was not practical. The anhydride precursor (**3.28**) is difficult to make and its reaction with $Ph_3P{=}CHCO_2Et$ gives rise to the undesired regioisomer[3.07] (**3.29**) (SCHEME 3.10).

SCHEME 3.10



3.28 **3.29**

Therefore, the required enollactone (**3.19**) was prepared via the reaction sequence depicted in pathway b of SCHEME 3.06[3.07]. Acid chloride (**3.14**) and $Ph_3P{=}CHCO_2Et$ (2 equivalent) were dissolved in CH_2Cl_2 and stirred at 0 °C for 1h and then 20 °C for 4h. The solvent was evaporated under reduced pressure and the crude oxazolidinone (**3.17**) was purified by radial chromatography.

Selective hydrolysis was achieved when the oxazolidinone (**3.17**), dissolved in methanol, was treated with 1N NaOH (10 equivalent). After 4h at 20 °C, the solution was acidified with 1N HCl, the solvent was evaporated under reduced pressure and the free acid (**3.18**) was extracted from the residue with ethyl acetate. The acid (**3.18**) was dissolved in $CHCl_3$ and refluxed for 48h. Radial chromatography led to the isolation of enollactone (**3.19**) in an overall yield of 66% from the acid chloride (**3.14**). It was necessary to perform the radial chromatography as rapidly as possible, because the enollactone (**3.19**) was unstable on silica.

Keto-amide (**3.20**) was obtained following reaction of enollactone (**3.19**), glycine ethylester hydrochloride (1.2 equivalent) and triethylamine (1.2 equivalent) in CH_2Cl_2 at 20 °C for 16h. The target E-enamino ester (**3.04**) was obtained from the keto-amide (**3.20**) on refluxing with PTSA in 1, 2-dichloroethane, with azeotropic removal of H_2O, for 4h. Overall, the enamino ester (**3.04**) was synthesized in a yield of 33% from the acid chloride (**3.14**) via the insertion route (pathway b, SCHEME 3.06). The β-keto ester route (pathway a, SCHEME 3.05) represented a higher yielding route (42%) with one less transformation.

SECTION 3.3.3

OPTICAL ACTIVITY OF ENAMINO ESTER (**3.04**)

The chiral centre of the starting compound (S)-CBz aspartic acid (**3.12**) should not be racemized by any of the reactions depicted in SCHEME 3.06. Therefore, enamino ester (**3.04**) is expected to be optically active, whether synthesized via the β-keto ester (pathway a) or insertion (pathway b) route. The specific rotation of enamino ester (**3.04**) was measured to be $(\alpha)_D^{20} = -13°$ (c 0.35; CH_2Cl_2). More evidence that the reactions do not racemize the chiral centre is discussed in Chapter 4 (*Section 4.5.3*).

SECTION 3.4

CHAPTER 3 REFERENCES

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3.02 Cason, J. *Organic Syntheses, Coll. Vol. III* John Wiley and Sons, New York, **1955**; 169.

3.03 a) Oikawa, Y.; Sugano, K.; Yonemitsu, O. *J. Org. Chem.* **1978**, *43*, 2087.

b) Oikawa, Y.; Yoshioka, T.; Sugano, K.; Yonemitsu, O. *Organic Syntheses Coll. Vol. VII* John Wiley and Sons, New York, **1990**; 359.

3.04 Hickmott, P. W. *Tetrahedron* **1982**, *38*, 1975.

3.05 Scholtz, J. M.; Bartlett, P. A. *Synthesis* **1989**, 542.

3.06 Hashiguchi, S.; Natsugari, H.; Ochiai, M. *J. Chem. Soc. Perkin Trans. 1* **1988**, 2345.

3.07 Trent, J. O. Ph.D. Thesis, University of Canterbury, **1992**.

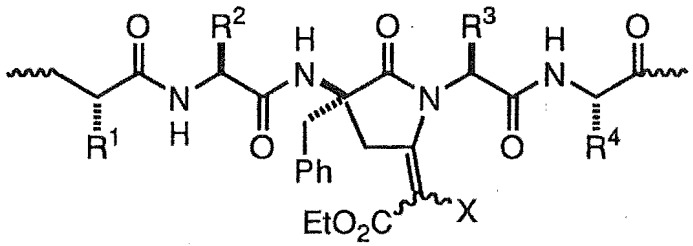
CHAPTER 4

SYNTHESIS OF THE TARGET 3,3-DISUBSTITUTED ENAMINO ESTERS VIA THE INSERTION REACTION AND FROM β-KETO ESTER

SECTION 4.1

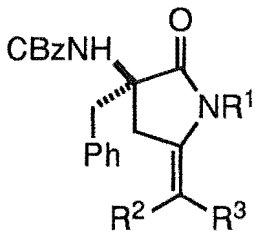
INTRODUCTION

This chapter describes the synthesis of 3,3-disubstituted bromo enamino esters (**4.01E** and **4.01Z**) and 3,3-disubstituted protio enamino esters (**4.02-4.07**) (TABLE 4.01). The bromo enamino esters (**4.01E** and **4.01Z**) are key intermediates for the target molecule (**i.34**) and represent a new class of potential mechanism-based inactivators of chymotrypsin (*Section i.5*, Introduction). The protio enamino esters (**4.02-4.07**) are key intermediates to the target molecule (**i.35**) and represent a new class of potential alternate substrate inhibitors of chymotrypsin (*Section i.5*, Introduction).



i.34 X = Br
i.35 X = H

TABLE 4.01



4.01 - 4.07

Compd	R^1	R^2	R^3
4.01E	CH_2CO_2Et	Br	CO_2Et
4.01Z	CH_2CO_2Et	CO_2Et	Br
4.02	CH_2CO_2Et	CO_2Et	H
4.03	$CH_2CONHCH_2CO_2Et$	CO_2Et	H
4.04	$CH_2CO_2{}^tBu$	CO_2Et	H
4.05	CH_2CO_2H	CO_2Et	H
4.06	(S)-$CH(Me)CO_2Et$	CO_2Et	H
4.07	^nBu	CO_2Et	H

The 3,3-disubstituted enamino esters (**4.01-4.07**), unlike the enamino esters described in Chapters 2 and 3, contain an amino acid R group; namely the benzyl group of phenylalanine, at position 3 which is required for recognition by the target enzyme. Methodology developed largely by Seebach and coworkers for the asymmetric synthesis of α,α-disubstituted amino acids[4.01] was extended to allow the introduction of the benzyl group, with stereo-control, into the potential inhibitors (**4.01-4.07**). The methodology developed in the current work should enable the introduction of any amino acid at position 3.

SECTION 4.2

BENZYLOXYCARBONYL (CBz) AND BENZOYL OXAZOLIDINONES

SECTION 4.2.1

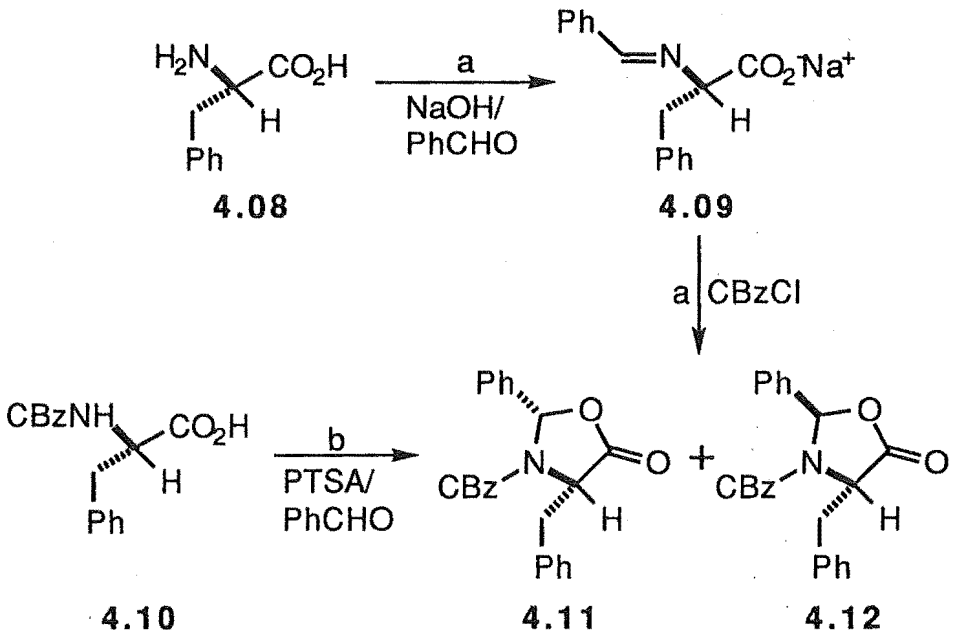
SYNTHESIS OF CBz OXAZOLIDINONES

SYNTHESIS OF 4-SUBSTITUTED CBz OXAZOLIDINONE (**4.11**)

Synthesis of the oxazolidinone precursors to enamino esters (**4.01-4.07**, TABLE 4.01) is summarized in SCHEMES 4.01 and 4.02. The key oxazolidinone (**4.11**) was prepared via the procedure reported by Seebach et al[4.02] (pathway a, SCHEME 4.01) and also via the procedure reported by Karady et al[4.03] (pathway b, SCHEME 4.01). The former method (pathway a, SCHEME 4.01) involved treatment of the Schiff base salt (**4.09**) of (S)-phenylalanine (**4.08**), in CH_2Cl_2, with benzyl chloroformate (1 equivalent) and the resulting mixture was stirred at -20 °C for 1 day and at 4 °C for 3 days. The latter method (pathway b, SCHEME 4.01) involved treatment of (S)-CBz phenylalanine (**4.10**), in 1,1,1-trichloroethane, with benzaldehyde (2 equivalent) and *p*-toluene sulphonic acid (PTSA) (1 equivalent). The solution was refluxed for 18h with azeotropic removal of H_2O. A ^1H NMR spectrum of the crude oxazolidinone (**4.11**) revealed the presence of less than 5% of the trans epimer (**4.12**) in the case of pathway a and 50% of the trans epimer (**4.12**) for pathway b (cis/trans assignment is discussed later in *Section 4.2.3*). The desired cis oxazolidinone (**4.11**) was

separated from its epimer (**4.12**) by silica column chromatography and recrystallization in an overall yield of 47% for pathway a, SCHEME 4.01 and 21% for pathway b, SCHEME 4.01.

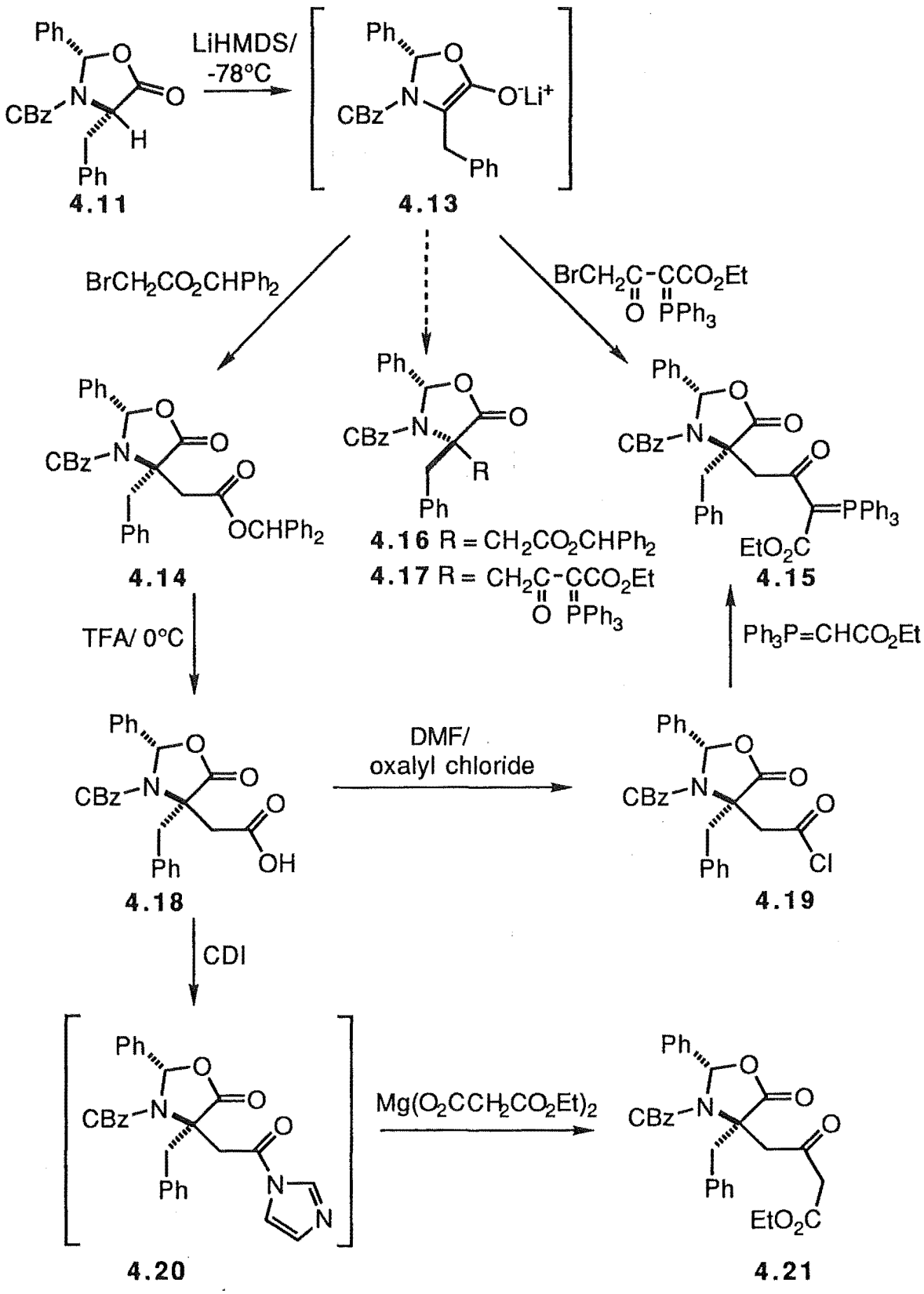
SCHEME 4.01



SYNTHESIS OF 4,4-DISUBSTITUTED CBz OXAZOLIDINONES

The synthesis of 4,4-disubstituted CBz oxazolidinones is summarized in SCHEME 4.02. Oxazolidinone (**4.11**) was alkylated, with high degree of diastereoselectivity, using the method pioneered by Seebach [4.01]; a THF solution of oxazolidinone (**4.11**), at -78 °C, was treated with a 1M THF solution of lithium hexamethyldisilazide (LiHMDS) (1.1 equivalent) and the resulting solution was stirred at -78 °C for 7min. The alkylating agent, $BrCH_2CO_2CHPh_2$ or $BrCH_2COC(PPh_3)CO_2Et$, was added and the solution was stirred at -78 °C for 2h, and subsequently at 20 °C for 16h. The crude oxazolidinones (**4.14** and **4.15**) contained less than 5% of the minor (2S,4R)-epimer (**4.16** and **4.17**, respectively), by ^1H NMR spectroscopy. Phosphorane oxazolidinone (**4.15**) was obtained in a yield of 26% after radial chromatography. The crude benzhydryl oxazolidinone (**4.14**), which was obtained quantitatively, was subsequently used without further purification.

SCHEME 4.02


LiHMDS/
-78°C
4.11
4.13
BrCH2CO2CHPh2
BrCH2C-CCO2Et
O PPh3
4.14
4.16 R = CH2CO2CHPh2
4.17 R = CH2C-CCO2Et
O PPh3
4.15
TFA/ 0°C
Ph3P=CHCO2Et
DMF/
oxalyl chloride
4.18
4.19
CDI
Mg(O2CCH2CO2Et)2
4.20
4.21

The formation of the 4,4-disubstituted oxazolidinones (**4.14** and **4.15**) from oxazolidinone (**4.11**) proceeds via the unstable planar enolate (**4.13**). The chiral centre of the enolate controls the stereochemical outcome of the alkylation reaction. The alkylating group approaches from the sterically least hindered face of the enolate; *ie* opposite the phenyl group. The configuration at this centre was dictated by the chiral centre in (S)-phenylalanine (**4.08**) or (S)-CBz phenylalanine (**4.10**). Overall the stereochemical outcome of the sequence is described as self reproduction of chirality because the formation of oxazolidinones (**4.14** and **4.15**) proceeded with retention of configuration (assignment of configuration is discussed later in *Section 4.2.3*).

The Seebach alkylation reaction has been carried out with oxazolidinones derived from most amino acids and with a number of alkylating agents and bases; for example, LiHMDS, LDA and $LiN(Et)_2$ [4.01]. The alkylation using the phosphorane $BrCH_2COC(PPh_3)CO_2Et$ represents a new example of this existing important strategy.

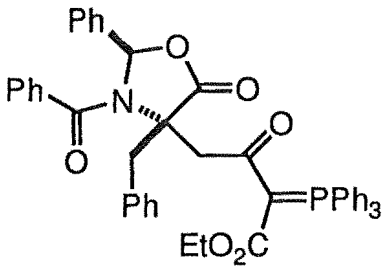
The benzhydryl group was removed on treatment of benzhydryl oxazolidinone (**4.14**) with TFA (trifluoroacetic acid) (20 equivalent), at 0 °C. The acid (**4.18**) was purified by recrystallization, to give a final yield of 32%. Attempts to purify the acid (**4.18**) by extraction into $NaHCO_3$ led to emulsification.

The acid (**4.18**) was dissolved in CH_2Cl_2, cooled to 0 °C and treated with oxalyl chloride (5 equivalent) and a catalytic quantity of DMF to give the acid chloride (**4.19**), in a yield of 100%.

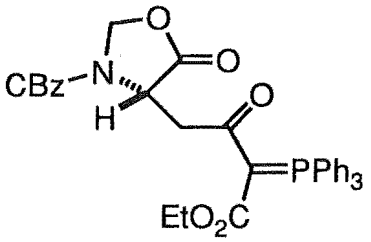
Treatment of the acid chloride (**4.19**) with $Ph_3P{=}CHCO_2Et$ (2 equivalent), in CH_2Cl_2, at 0 °C for 1.5h and at 20 °C for 4.5h, followed by radial chromatography, quantitatively gave the phosphorane (**4.15**). Therefore, although requiring three additional steps, the phosphorane (**4.15**) was prepared in superior yield and purity, by ^1H NMR spectroscopy, via this method, rather than via alkylation of the oxazolidinone (**4.11**) with $BrCH_2COC(PPh_3)CO_2Et$.

The phosphorane (**4.15**) prepared via both routes was present as a 1 : 1 mixture, by ^1H NMR spectroscopy, of two conformers. The related benzoyl phosphorane (**4.26**, SCHEME 4.04) was present as a single isomer by ^1H and ^{13}C NMR spectroscopy. Therefore, it is likely that the conformational isomerism observed for CBz-phosphorane

(**4.15**) arises due to restricted rotation about the CBz group. The ^{1}H NMR spectra of CBz phosphorane (**4.15**) and benzoyl phosphorane (**4.26**) are shown; Spectrum 4.01 and 4.02, respectively. Resonances in the ^{1}H NMR spectrum of CBz phosphorane (**4.15**) (Spectrum 4.01) are doubled; for example there are two resolved triplets (inset) arising from the methyl groups, whereas the spectrum of benzoyl phosphorane (**4.26**) (Spectrum 4.02) has one triplet (inset). The ^{1}H and ^{13}C NMR spectra of the related phosphorane (**3.18**) indicated a single isomer; however, the ^{1}H NMR resonances were broad. The ^{1}H NMR spectrum of CBz-phosphorane (**4.15**) in d_6-DMSO, heated to 85 °C, indicated that a single conformer was present. The ^{1}H NMR spectra of CBz phosphorane in d_6-DMSO at 23 °C and 85 °C are shown; Spectrum 4.03 and 4.04, respectively. Again, doubling of resonances is evident in the ^{1}H NMR spectrum of CBz phosphorane (**4.15**) at 85 °C (Spectrum 4.03); for example there are two overlapping triplets (inset) arising from the methyl groups, whereas at 23 °C (Spectrum 4.04) there is one triplet (inset).



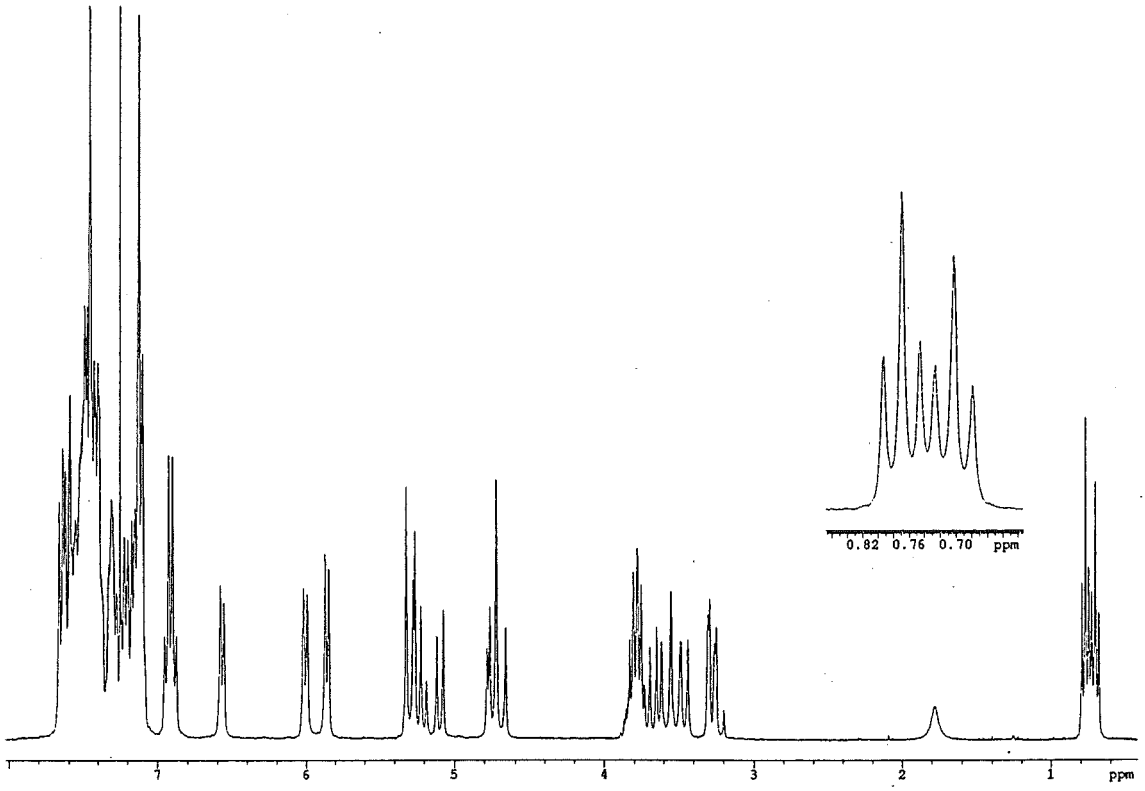
4.26



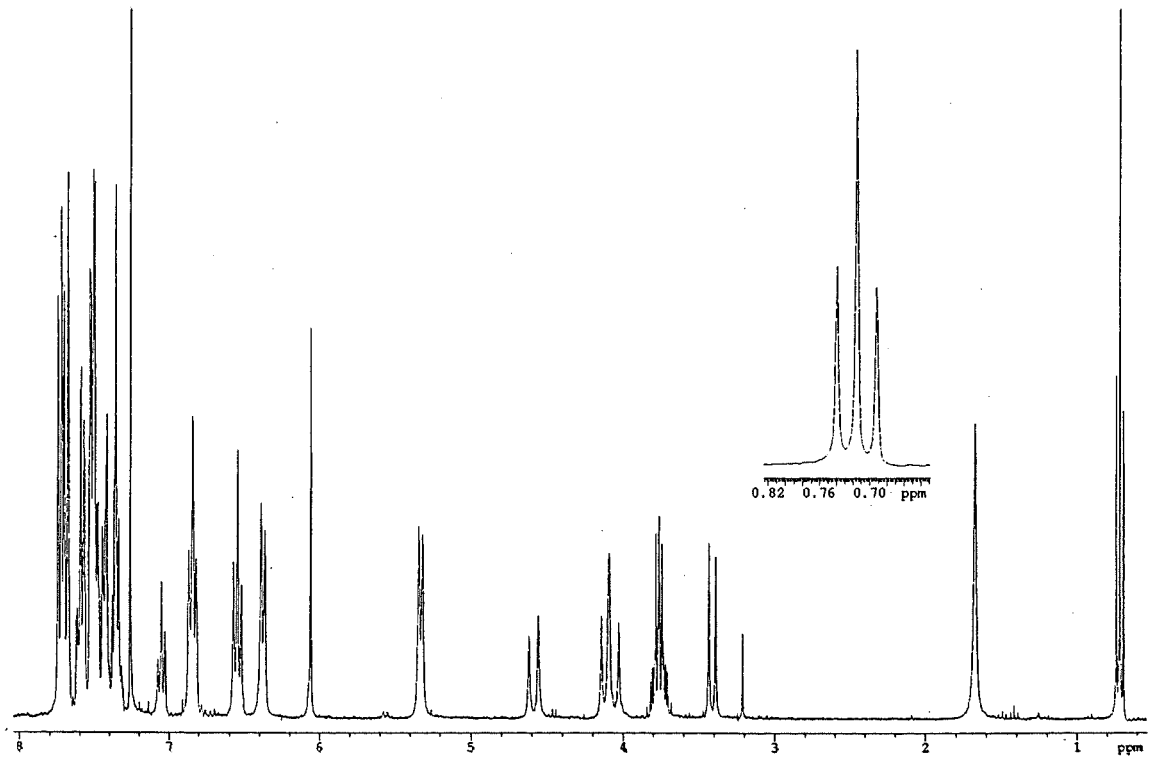
3.17

On treatment with carbonyl diimidazole (CDI) (1.2 equivalent) in THF, the acid (**4.18**) was converted into the corresponding imidazole[4.04] (**4.20**) which, without isolation, was treated with magnesium diethyl malonate [4.05] (1 equivalent) to give the β-keto ester (**4.21**) in a yield of 65%, after chromatography.

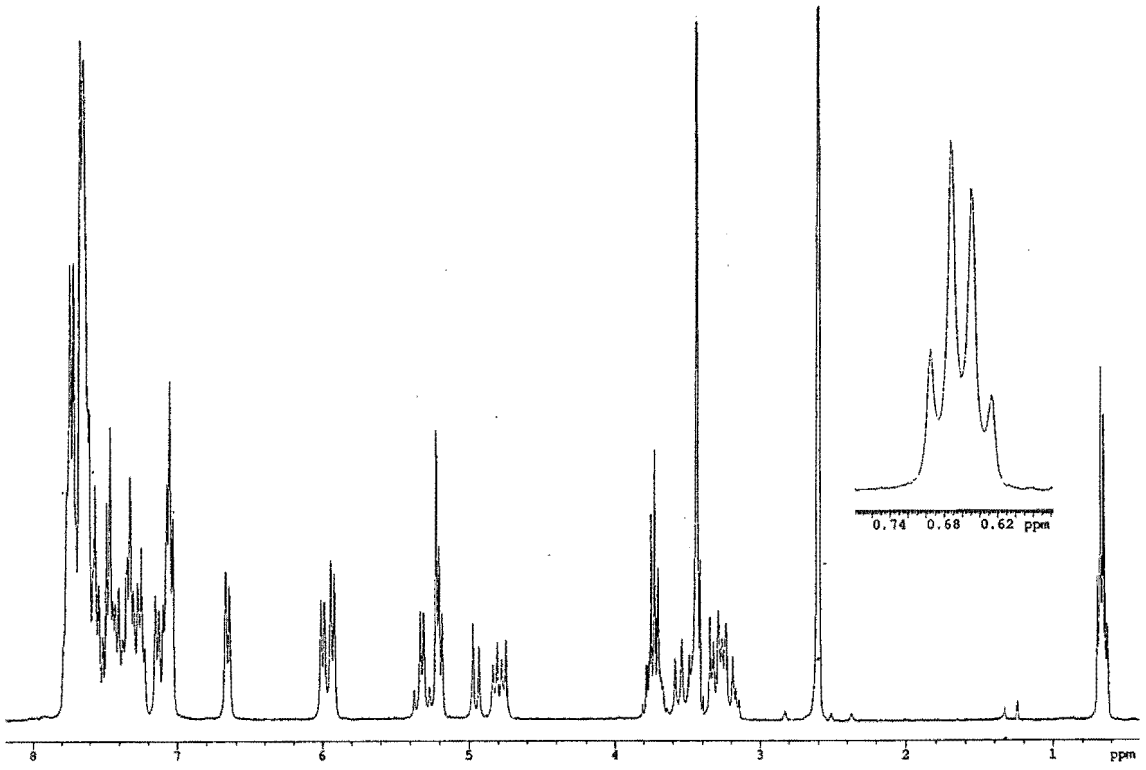
Spectrum 4.01



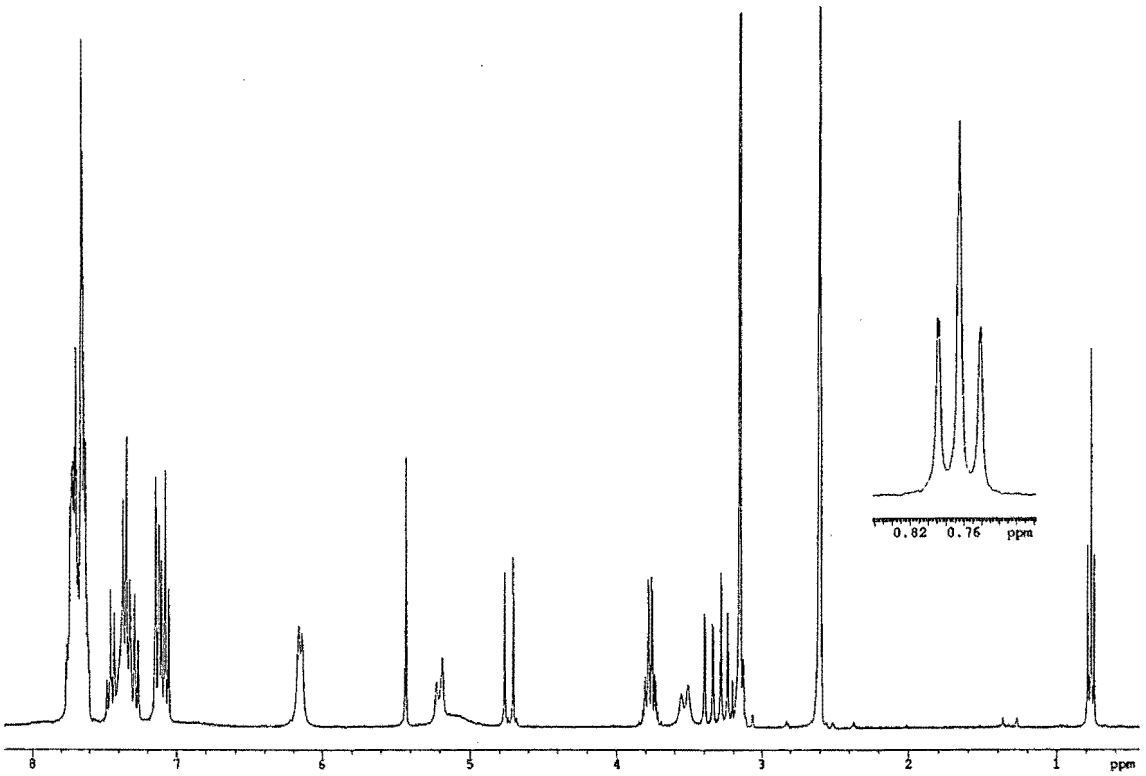
Spectrum 4.02



Spectrum 4.03



Spectrum 4.04



SECTION 4.2.2

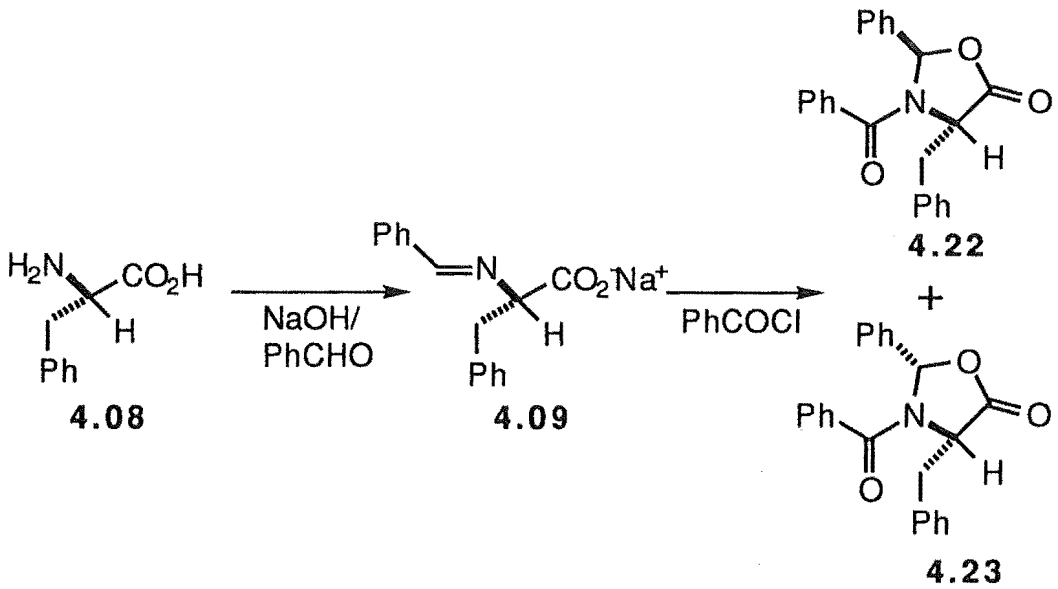
SYNTHESIS OF BENZOYL OXAZOLIDINONES

The analogous oxazolidinones derived from benzoyl oxazolidinone (**4.22**) were prepared using the general methods described above, in some cases with minor modifications (SCHEMES 4.03 and 4.04).

SYNTHESIS OF 4-SUBSTITUTED OXAZOLIDINONE (**4.22**)

Reaction of the Schiff base salt (**4.09**) of (S)-phenylalanine (**4.08**) with benzoylchloride (1 equivalent) at -20 °C for 1 day and at 4 °C for 4 days gave a mixture containing, by ^{1}H NMR spectroscopy, 70% trans oxazolidinone (**4.22**) and 30% cis oxazolidinone (**4.23**) (cis/trans assignment is discussed later in *Section 4.2.3*). The trans oxazolidinone (**4.22**) was purified by recrystallization, in a yield of 25%.

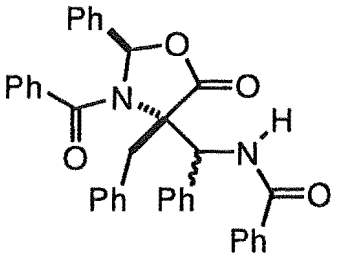
SCHEME 4.03



SYNTHESIS OF 4,4-DISUBSTITUTED OXAZOLIDINONES

The synthesis of 4,4-disubstituted benzoyl oxazolidinones is summarized in SCHEME 4.04. The enolate (**4.24**) was prepared by treatment of the oxazolidinone (**4.22**), dissolved in THF at -78 °C, with LiHMDS (SCHEME 4.04). Alkylation of the enolate (**4.24**, SCHEME 4.04) with $BrCH_2CO_2CHPh_2$ gave a mixture, used subsequently without further purification, which contained, by ^{1}H NMR spectroscopy, 67% of the desired benzhydryl

oxazolidinone (**4.25**), 22% $BrCH_2CO_2CHPh_2$ and 11% of a compound tentatively assigned as the dimer (**4.33**, discussed later in *Section 4.3*). ^1H NMR spectroscopy revealed that less than 5% of the minor diastereoisomer (**4.27**) had formed. Hence, overall the reactions leading to formation of oxazolidinone (**4.25**) proceeded with inversion of configuration.

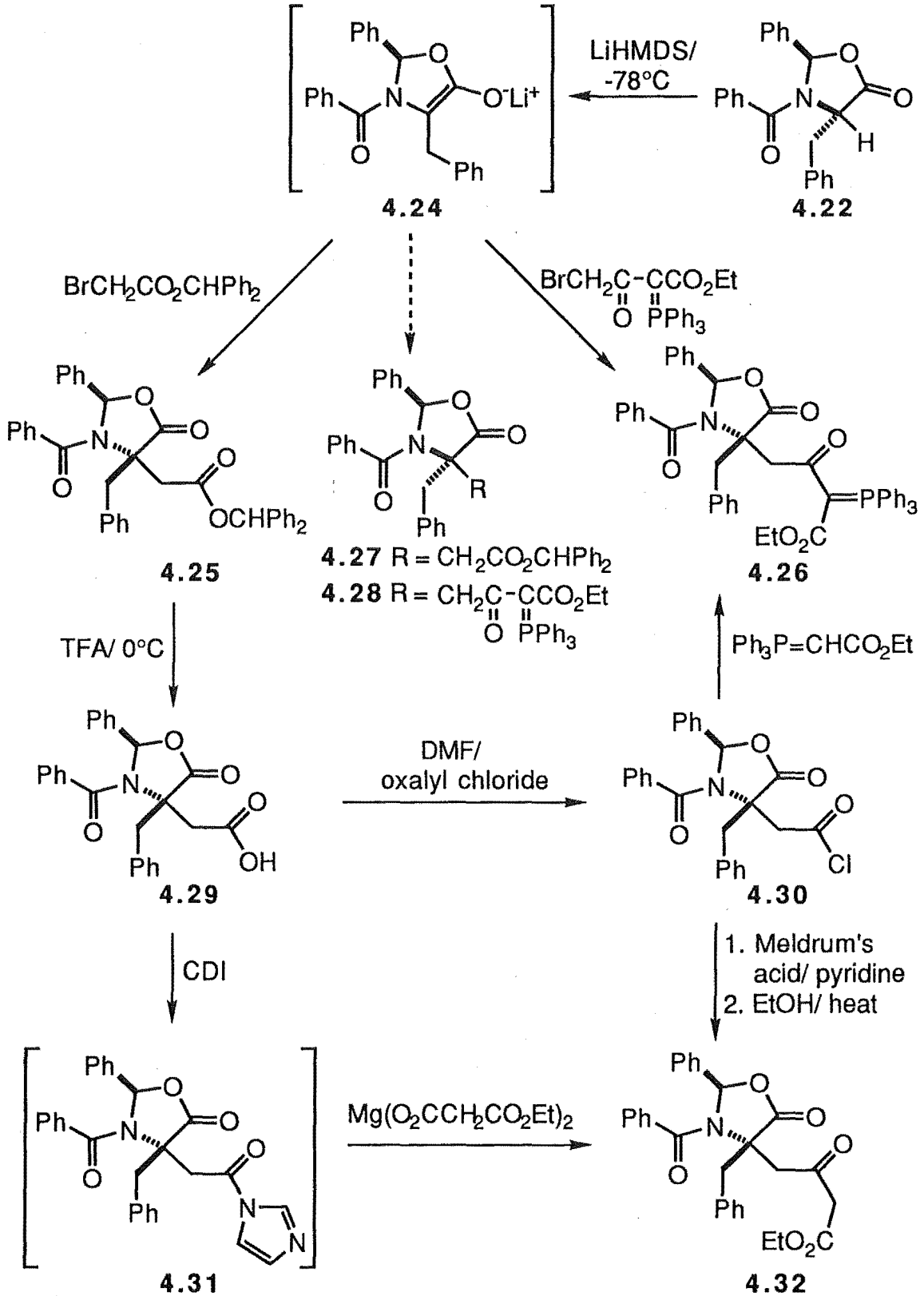


4.33

Alkylation of the enolate (**4.24**) with $BrCH_2COC(PPh_3)CO_2Et$ gave a mixture which contained, by ^1H NMR spectroscopy, 15% of the phosphorane oxazolidinone (**4.26**), 70% recovered $BrCH_2COC(PPh_3)CO_2Et$ and 15% of the dimer (**4.33**, discussed later in *Section 4.3*). Again, less than 5% of the minor diastereoisomer (**4.28**) was observed by ^1H NMR spectroscopy. Purification of phosphorane oxazolidinone (**4.26**) was not achieved by silica or diol chromatography. However, benzoyl phosphorane (**4.26**) was prepared in superior yield and purity from the acid (**4.29**) via reaction with oxalyl chloride and DMF followed by $Ph_3P{=}CHCO_2Et$ (SCHEME 4.04). The acid (**4.29**) was prepared via treatment of benzhydryl oxazolidinone (**4.25**) with TFA, and purified by an acid/base extraction followed by recrystallization.

Benzoyl β-keto ester (**4.32**) was prepared from the acid (**4.29**), in a yield of 91%, via reaction with carbonyl diimidazole[4.04] (CDI) followed by magnesium diethyl malonate[4.05]. In contrast, benzoyl β-keto ester (**4.32**) was also prepared, in 21% yield, via reaction of the acid (**4.29**) with oxalyl chloride and DMF followed by Meldrum's acid (2,2-dimethyl-1,3-dioxane-4,6-dione) and pyridine, then finally treatment with ethanol[4.07] (SCHEME 4.04). It is likely that the simpler β-keto esters (**3.01**, **3.14**) prepared via the Meldrum's acid route (Chapter 3, *Sections 3.2.1* and *3.2.3*), could have been better prepared via reaction of the appropriate acid with carbonyl diimidazole (CDI) and magnesium diethyl malonate.

SCHEME 4.04


LiHMDS/
-78°C
4.24
4.22
BrCH2CO2CHPh2
BrCH2C-CCO2Et
O PPh3
4.25
4.27 R = CH2CO2CHPh2
4.28 R = CH2C-CCO2Et
O PPh3
4.26
TFA/ 0°C
Ph3P=CHCO2Et
DMF/
oxalyl chloride
4.29
4.30
CDI
1. Meldrum's
acid/ pyridine
2. EtOH/ heat
Mg(O2CCH2CO2Et)2
4.31
4.32

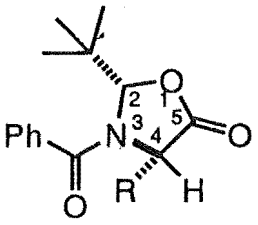
SECTION 4.2.3

ASSIGNMENT OF R/S CONFIGURATION TO THE OXAZOLIDINONES

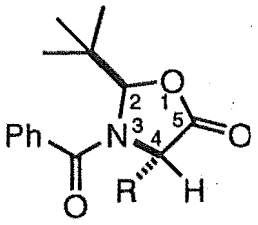
The benzoyl oxazolidinone (**4.22**, SCHEMES 4.03 and 4.04) was assigned the trans configuration on the basis of melting point and IR, ^{1}H NMR and ^{13}C NMR data identical to that reported[4.06] (**4.22**). Assignment of the trans configuration was confirmed by the observation of an n.O.e. between **H-2** and CH_2Ph.

Literature[4.06] assignment of the trans configuration to (**4.22**) was based on comparison of the chemical shifts of the protons at **H-2** and **H-4** with the corresponding chemical shifts of cis and trans 2-(*tert*-butyl) oxazolidinones[4.02, 4.08] (**4.34** and **4.35**, respectively) The **H-2** and **H-4** resonances, summarized in TABLE 4.02, are downfield in the trans isomer (**4.35**) relative to the corresponding cis isomer (**4.34**).

TABLE 4.02



4.34



4.35

	CIS			TRANS		
	Compd			Compd		
R	**4.34**	δ H-2	δ H-4	**4.35**	δ H-2	δ H-4
Me	a	6.13	4.07	a	6.25	4.37
$CHMe_2$	b	5.95	4.29	b	6.14	4.29
CH_2CH_2SMe	c	6.10	4.20	c	6.21	4.52
$(CH_2)_4NHCO_2{}^{t}Bu$	d	6.05	3.88	d	6.16	4.33-4.46
	4.23	*	4.92	**4.22**	5.83	5.2
	4.11	6.45	4.66	**4.12**	*	4.71

* Sample of insufficient purity to assign δ H-2

CBz oxazolidinone (**4.11**) was assigned the cis configuration on the basis of IR and ^{13}C NMR data identical to that reported[4.03]. There is some ambiguity regarding the configuration because, while the text reports that the cis isomer (**4.11**) was obtained from (S)-phenylalanine, the figures depict (R)-phenylalanine giving rise to the corresponding cis isomer[4.03]. The configuration of the cis oxazolidinone (**4.11**) was reportedly confirmed by single crystal X-ray structure analysis[4.03]. The atom coordinates of the X-ray analysis were obtained from the Cambridge crystallographic data base; however, due to anomalies in the hydrogen coordinates, the configuration was not able to be unambiguously determined.

On the basis of the upfield position of the **H-4** resonance in the major isomer relative the minor isomer, the major isomer was tentatively assigned the cis configuration (**4.11**) (TABLE 4.02). An n.O.e. was not observed between **H-2** and **H-4** or C4C**H**$_2$Ph.

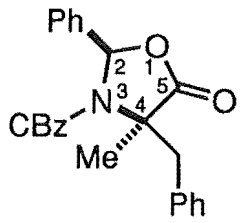
The configuration of 4,4-disubstituted benzoyl oxazolidinones (**4.25-4.26, 4.29-4.32**, SCHEME 4.04) was confirmed by the observation of an n.O.e. between **H-2** and C**H**$_2$Ph in the acid (**4.29**). This confirmed that the electrophilic alkylations had occurred from the least sterically hindered face of the enolate (**4.24**).

The configuration of the 4,4-disubstituted CBz oxazolidinones (**4.14-4.15, 4.18-4.21**, SCHEME 4.02) was confirmed by the observation of an n.O.e. between **H-2** and C4C**H**$_2$CO in the β-keto ester (**4.21**). In all reported alkylations[4.01] the electrophile attacked the least sterically hindered face of the enolate. Therefore, it is likely that the oxazolidinone (**4.11**), a precursor of the alkylated (2S,4R)-oxazolidinones (**4.14-4.15, 4.18-4.21**, SCHEME 4.02), has the cis configuration.

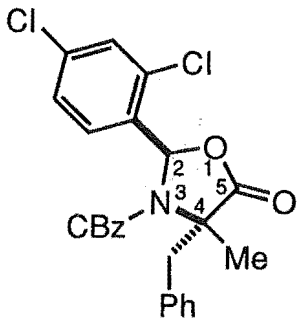
SECTION 4.2.4

TRENDS IN THE ^{1}H NMR, ^{13}C NMR AND HIGH RESOLUTION MASS SPECTRA OF OXAZOLIDINONES

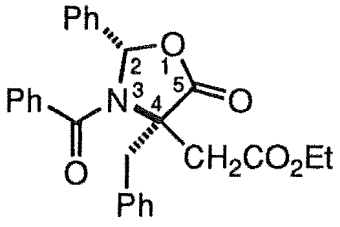
The ^{1}H and ^{13}C NMR spectral data of oxazolidinones (**4.11**, **4.14-4.15**, **4.18-4.19**, **4.21-4.22**, **4.25-4.26**, **4.30**, **4.32**, SCHEMES 4.01-4.04) and of literature oxazolidinones[4.02-4.03] (**4.36-4.39**) are summarized in TABLE 4.03. ^{13}C NMR resonances for **C-2**, **C-4**, C4CH_2Ph, **C-3**, PhCN and PhCH_2OCN appeared at characteristic chemical shifts for all examples. In the ^{1}H NMR spectra, **H-2** characteristically resonated at δ 5.29-6.51. In the alkylated oxazolidinones (**4.14-4.15**, **4.18-4.19**, **4.21**, **4.25-4.26**, **4.30**, **4.32**) C4CH_2Ph and C4CH_2R appeared as AB quartets with J=13Hz and J=18Hz, respectively. In the CBz oxazolidinones (**4.11**, **4.14-4.15**, **4.18-4.19**, **4.21**) OCH_2Ph also appeared as an AB quartet, typically with J=12Hz.



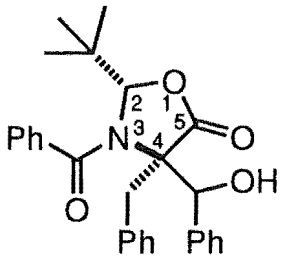
4.36



4.37



4.38



4.39

The mass spectra of the oxazolidinones (**4.11**, **4.14-4.15**, **4.18-4.19**, **4.21-4.22**, **4.25-4.26**, **4.30**, **4.32**, SCHEMES 4.01-4.04) characteristically showed a large signal, often the base peak, at M-91 corresponding to M-CH_2Ph.

TABLE 4.03: Characteristic ^{1}H and ^{13}C NMR Resonances of Oxazolidinones

						C4CH_2Ph (AB_q)			C4CH_2R (AB_q)			OCH_2Ph (AB_q)		
No.	C-2	C-4	C4CH_2	CON	δ H-2	δ H_a	δ H_b	J_{AB} Hz	δ H_a	δ H_b	J_{AB} Hz	δ H_a	δ H_b	J_{AB} Hz
4.11	82.2	58.3	36.6	153.9	6.45	3.19-3.43*			N/A			5.05	5.16	12.1
4.14	^{13}C NMR not recorded				5.95	3.25	3.56	13.2	3.19	3.89	17.4	4.66	5.02	12.7
4.15●	89.4	65.2	41.9	151.7	5.29	3.27	3.67	13.4	3.51	4.761	18.0	4.74	5.09	12.2
	and	and	and	and	and		and			and			and	
	89.5	65.7	42.7	152.2	5.33	3.29	3.46	13.2	3.59	4.69	18.6	5.21	5.29	12.2
4.18	90.6	65.0	38.9	152.2	6.30	3.25	3.87	13.5	3.13	3.57	18.1	4.82	5.11	12.2
4.19	^{13}C NMR not recorded				6.30	3.20	3.50	13.2	3.61	4.37	19.1	4.84	5.10	12.2
4.21	90.4	64.3	41.7	152.2	6.38	3.20	3.52	13.2	3.28	4.10	18.8	4.79	5.05	12.2
4.22	91.3	57.8	34.9	169.3	5.83	3.4*			N/A					
4.25	^{13}C NMR not recorded				6.13	3.41	4.02	13.3	3.34	4.16	17.6			
4.26	90.7	67.7	42.9	169.0	6.06	3.41	4.12	13.5	4.05	4.59	18.5			
4.29	91.7	66.1	38.7	170.3	6.45	3.42	4.04	13.4	3.26	4.14	18.0			
4.30	^{13}C NMR not recorded				6.39	3.38	3.98	13.4	3.76	4.65	19.1			
4.32	91.6	65.4	42.3	170.0	6.49	3.37	3.99	13.4	3.41	4.39	19.0			
4.36	88.5	61.7	41.8	153.2	^{1}H NMR not reported									
4.37	85.1	64.9	40.9	151.7	^{1}H NMR not reported									
4.38	91.6	66.2	39.1	169.8	6.51	3.71	AB_q	13.5	3.61	AB_q	17.5			
4.39	89.9	69.4	39.3	165.1	6.12	3.11	AB_q	14	N/A					

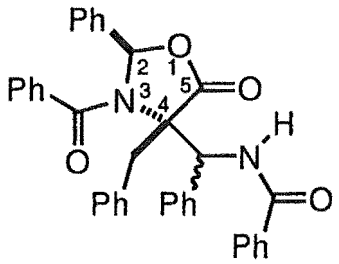
* ^{1}H NMR resonance overlapped with other resonances

● ^{1}H and ^{13}C NMR resonances are given for both conformers

SECTION 4.3

BENZOYL DIMER

Formation of the compound, tentatively assigned as the dimer (**4.33**), competed with electrophilic alkylation of the benzoyl enolate (**4.24**, SCHEME 4.04) by $BrCH_2CO_2CHPh_2$ or $BrCH_2COC(PPh_3)CO_2Et$. For the more sterically hindered electrophile; $BrCH_2COC(PPh_3)CO_2Et$, the reaction leading to formation of the dimer (**4.33**) was more favoured (*Section 4.2.2*).



4.33

Dimer (**4.33**) was also prepared, in 62% yield after chromatography, when LiHMDS (1.1 equivalent) and oxazolidinone (**4.22**) were dissolved in THF and stirred at -78 °C for 2h followed by 20 °C for 16h.

The dimer (**4.33**) is likely to form via a mechanism involving self condensation of oxazolidinone (**4.22**). Other examples of self condensation of oxazolidinones have been reported[4.09].

The structure of the dimer (**4.33**) was determined primarily by ^1H and ^{13}C NMR spectroscopy. The ^{13}C NMR spectrum of the dimer (**4.33**) showed resonances at δ 90.7, 74.6 and 38.1 which are characteristic of **C-2**, **C-4** and **C**H_2Ph, respectively (TABLE 4.03) and therefore reflect that (**4.33**) is an oxazolidinone. This was supported by the ^1H NMR spectrum which had resonances at δ 4.72 (s, 1H) and 3.61, 4.46 (AB_q, J_{AB}=13.9Hz) which are characteristic of **H-2** and C**H$_2$**Ph, respectively (TABLE 4.03). The remaining fragment was determined as containing two aromatic groups (from the ^1H and/or ^{13}C NMR spectra), NH (from the ^1H NMR spectrum), a carbonyl group (from the ^{13}C NMR spectrum) and a methine proton (from the ^1H and ^{13}C NMR spectra). Consideration of the chemical shifts and the connectivity of the oxazolidinone (**4.22**) from which the fragment is derived led to

proposal of the dimer (**4.33**). The ^{1}H and ^{13}C NMR assignments were supported by an NMR proton-carbon heteronuclear correlation experiment. The mass spectrum, IR spectrum and combustion analysis were also consistent with the proposed structure (**4.33**). The absolute stereochemistry about C4C*HPh is unknown. An X-ray crystal structure analysis, planned for the future, will unambiguously determine the structure of the compound (**4.33**).

SECTION 4.4

SYNTHESIS OF THE TARGET ENAMINO ESTERS

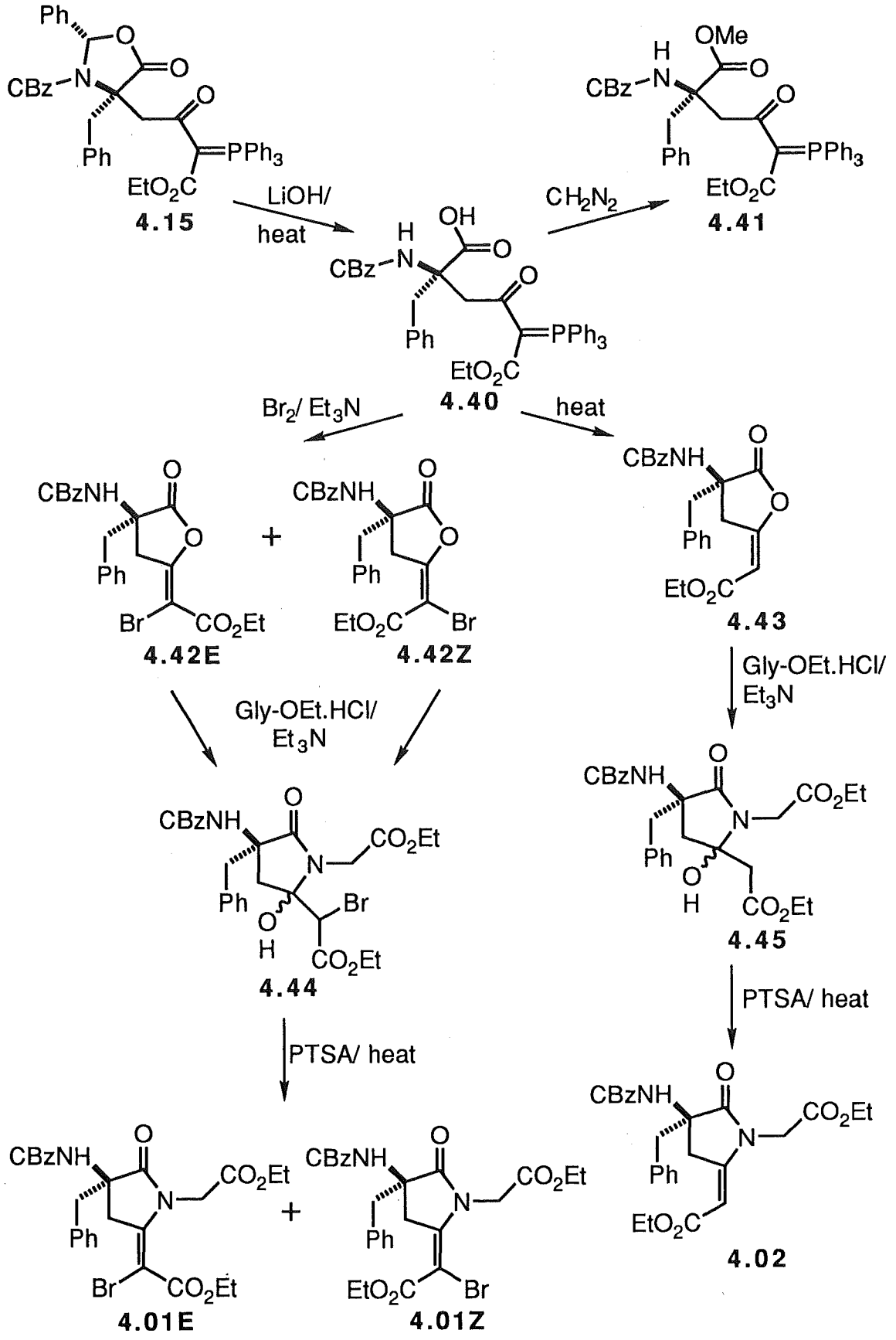
The target enamino esters (**4.01-4.06**, SCHEMES 4.05 and 4.07) were synthesized from CBz phosphorane oxazolidinone (**4.15**) via the insertion reaction (discussed in detail in Chapter 2) and the target enamino esters (**4.02** and **4.07**, SCHEME 4.08) were synthesized from CBz β-keto ester oxazolidinone (**4.21**) via the enamine route (discussed in detail in Chapter 3). The benzoyl oxazolidinones (**4.25** and **4.29**, SCHEME 4.02) were not used to prepare the corresponding benzoyl enamino esters.

SECTION 4.4.1

SYNTHESIS OF TARGET PROTIO AND BROMO ENAMINO ESTERS VIA THE INSERTION REACTION

Hydrolysis of the phosphorane oxazolidinone (**4.15**), dissolved in a mixture of THF and methanol, by reflux with 3.33N LiOH (103 equivalent) for 4h (SCHEME 4.05) gave the keto acid phosphorane (**4.40**) quantitatively. The related phosphorane oxazolidinone (**3.18**) was hydrolyzed by the less harsh conditions of treatment with 1N NaOH (10 equivalent) at 20 °C for 4h, in methanol[4.10] (*Section 3.3.2*, Chapter 3). The keto acid phosphorane (**4.40**) was relatively unstable and was used subsequently without further purification. However, methylation with CH_2N_2, followed by radial chromatography, gave the corresponding methyl ester (**4.41**) which was fully characterized. The acid (**4.40**) and methyl ester (**4.41**) existed as single conformers, unlike the oxazolidinone (**4.15**, *Section 4.2.1*).

SCHEME 4.05


Ph
CBz
N
O
Ph
PPh3
EtO2C
4.15
LiOH/
heat
CH2N2
OMe
H
OH
4.41
4.40
Br2/ Et3N
heat
CBzNH
Br
CO2Et
EtO2C
4.42E
4.42Z
4.43
Gly-OEt.HCl/
Et3N
4.44
4.45
PTSA/ heat
4.01E
4.01Z
4.02

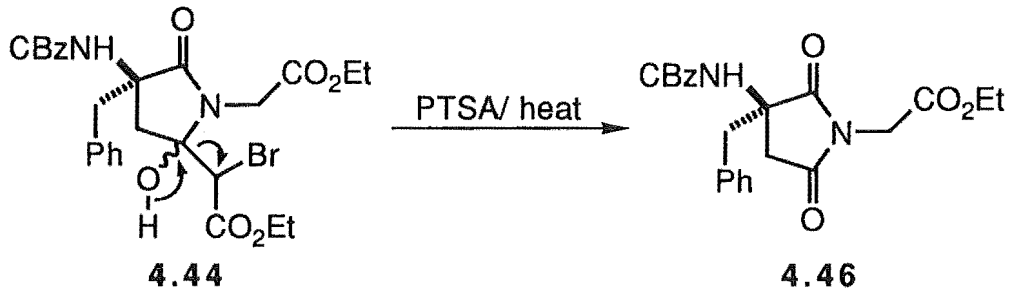
The keto acid phosphorane (**4.40**) was refluxed in THF for 6h to give the protio enollactone (**4.43**) which was isolated in a yield of 73% following radial chromatography (SCHEME 4.05). The corresponding E- and Z-bromo enollactones (**4.42E** and **4.42Z**, respectively) were formed via bromo enol-lactonization of the keto acid phosphorane (**4.40**) (SCHEME 4.05). Halo enol-lactonization of keto-acid phosphoranes is discussed in detail in Chapter 1. Thus, a CH_2Cl_2 solution of the acid (**4.40**), Br_2 (1 equivalent) and triethylamine (1 equivalent) was stirred at 0 °C for 20min and at 20 °C for 30min. The solvent was evaporated under reduced pressure to give a residue which contained, by ^1H NMR spectroscopy, E and Z isomers in a ratio of 46% E (**4.42E**) : 54% Z (**4.42Z**). The isomers were separated by radial chromatography to give a combined yield of 68%.

The protio enollactone (**4.43**) and bromo enollactone (**4.42E** or **4.42Z**) were dissolved in CH_2Cl_2 and stirred for 16h at 20 °C with glycine ethylester hydrochloride (2-3 equivalent) and triethylamine (2-3 equivalent) to yield the corresponding acylated amino alcohols (**4.45** and **4.44**, respectively, SCHEME 4.05). For the less substituted succinimide series discussed in Chapters 2 and 3, the acyclic keto-amide intermediate (for example **2.36**, SCHEME 2.08, *Section 2.1*, Chapter 2) was isolated. In the case of (**4.44**) and (**4.45**) increased substitution promotes cyclization via the "gem-dimethyl effect" (also known as the "Thorpe-Ingold effect")[4.11] and consequently the acylated amino alcohols (**4.44** and **4.45**) were isolated as the reaction intermediates. Protio hydroxy lactam (**4.45**) contains 2 chiral centres and was observed, by ^1H NMR spectroscopy, to exist as a mixture of 2 diastereoisomers in a ratio of 9 : 1. Bromo hydroxy lactam (**4.44**) contains 3 chiral centres and was observed by ^1H NMR spectroscopy to exist as a complex mixture of diastereoisomers.

The protio enamino ester (**4.02**) and bromo enamino esters (**4.01E** and **4.01Z**) were formed when the protio (**4.45**) and bromo (**4.44**) acylated amino alcohols, respectively, were dissolved in 1, 2-dichloroethane, containing PTSA, and refluxed for 3-3.5h with azeotropic removal of H_2O. The crude enamino esters were purified by radial chromatography to give E-protio enamino ester (**4.02**) in a yield of 68% and an inseparable mixture of E- and Z-bromo enamino esters (**4.01E** and **4.01Z**, respectively) in a combined yield of 65% and in the ratio of 15% E (**4.01E**) : 85% Z (**4.01Z**), by ^1H NMR

spectroscopy. The bromo enamino esters (**4.01**) were prepared in identical yield and isomer ratio from reaction of glycine ethylester with E-bromo enollactone (**4.42E**) and Z-bromo enollactone (**4.42Z**). This observation is consistent with the insertion reaction mechanism shown in SCHEME 2.20 (*Section 2.5*, Chapter 2). The corresponding imide (**4.46**, SCHEME 4.06) was isolated from the crude bromo enamino ester reaction mixture in 13% yield. It is likely that the imide (**4.46**) forms from the hydroxy lactam (**4.44**) via a retro-Claisen type reaction as discussed in *Section 2.7*, Chapter 2 and summarized in SCHEME 4.06. We expect that the yield of bromo enamino esters (**4.01E** and **4.01Z**) could be elevated by acetate formation with 4-DMAP (discussed in *Section 2.7.1*, Chapter 2).

SCHEME 4.06



4.44 **4.46**

The 3,3-disubstituted bromo enamino esters (**4.01E** and **4.01Z**) were stable, unlike the less substituted bromo and chloro enamino esters (*Sections 2.7.1-2.7.2*, Chapter 2 and *Section 3.2.3*, Chapter 3), because the competing elimination reaction was blocked by 3,3-disubstitution.

The protio enollactone (**4.43**) and bromo enollactones (**4.42E** and **4.42Z**) from which the bromo and protio enamino esters (**4.01** and **4.02**, respectively) were derived, may be inhibitors of chymotrypsin in their own right (*Section I.3.1* and *I.3.2*, Introduction); .

SECTION 4.4.2

SYNTHESIS OF OTHER PROTIO ENAMINO ESTERS VIA THE INSERTION REACTION

Synthesis of the target enamino esters (**4.03-4.06**) is summarized in SCHEME 4.07. The protio enollactone (**4.43**), glycylglycine ethylester hydrochloride (5.4 equivalent) and triethylamine (5.4 equivalent) in 1, 2-dichloroethane were refluxed for 44h with azeotropic removal of H_2O. PTSA was added and the mixture was refluxed for a further 4h with azeotropic removal of H_2O. Purification by radial chromatography gave E-enamino ester (**4.03**) in a yield of 64%.

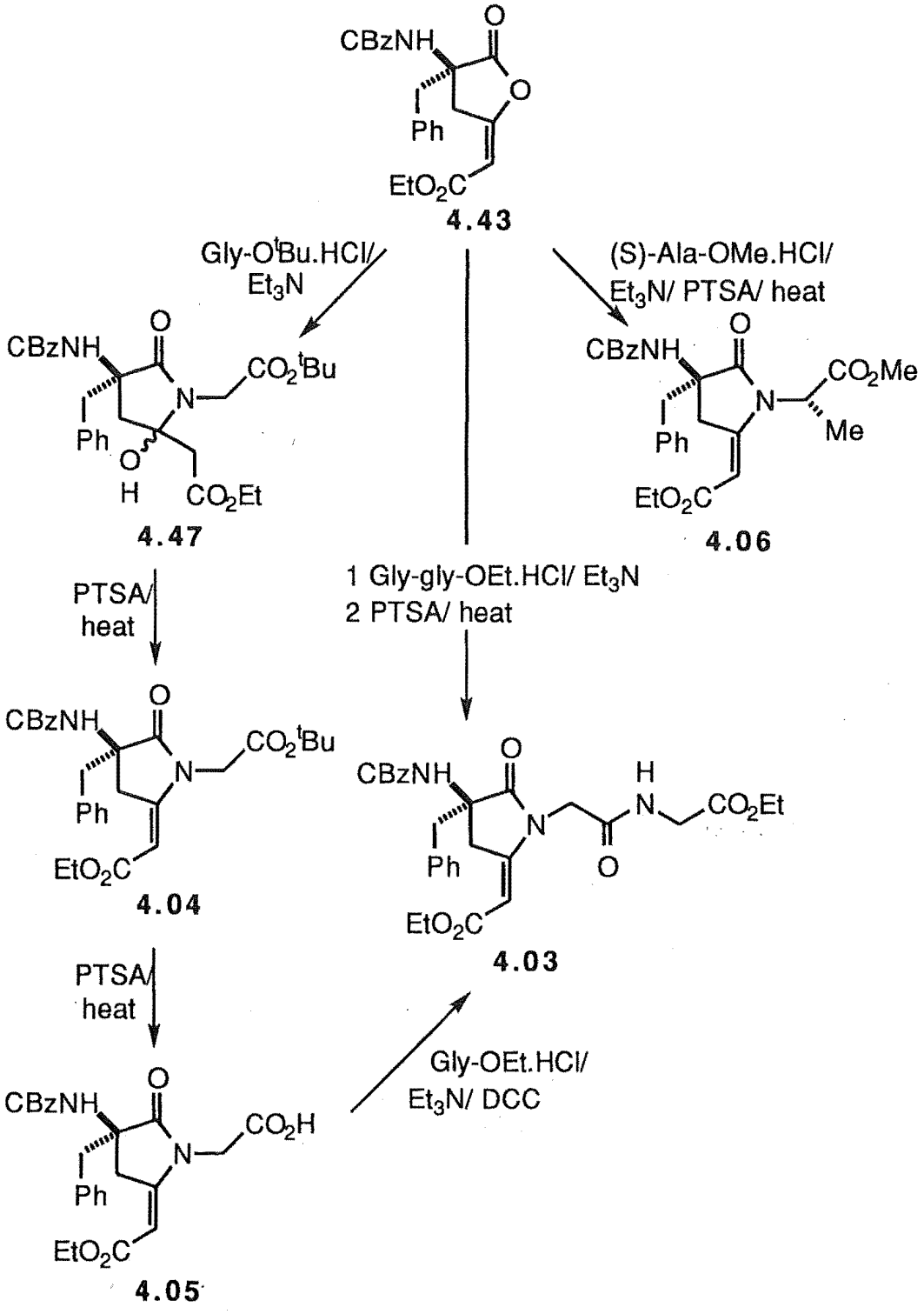
E-enamino ester (**4.03**) was also prepared, in the reduced yield of 51%, via the stepwise addition of glycine units. This demonstrates the feasibility of incorporating an inhibitor into an oligopeptide specific for the target enzyme. Reaction of protio enollactone (**4.43**) with *tert*-butyl glycine ethylester hydrochloride (2 equivalent) and triethylamine (2 equivalent) in CH_2Cl_2 at 20 °C gave the corresponding hydroxy lactam (**4.47**) as a mixture of diastereoisomers in the ratio of 9 : 1, by ^1H NMR spectroscopy.

The hydroxy lactam (**4.47**) was dissolved in 1, 2-dichloroethane containing PTSA and refluxed for 3h, with azeotropic removal of H_2O, to give the *tert*-butyl E-enamino ester (**4.04**). More PTSA was added to *tert*-butyl E-enamino ester (**4.04**), dissolved in benzene, and the solution was refluxed, with azeotropic removal of H_2O, for 3h to yield the deprotected E-enamino ester (**4.05**). Finally, E-enamino ester (**4.05**), DCC (1,3-dicyclohexylcarbodiimide) (1 equivalent), glycine ethylester hydrochloride (1.1 equivalent) and triethylamine (1.1 equivalent) in CH_2Cl_2 were stirred at 20 °C for 16h to form crude E-enamino ester (**4.03**) which was purified by radial chromatography.

Protio enollactone (**4.43**), (S)-alanine methylester hydrochloride (15 equivalent) and triethylamine (15 equivalent), in 1, 2-dichloroethane, were refluxed for 43h with azeotropic removal of H_2O to give crude E-enamino ester (**4.06**) which was isolated in a yield of 78% following radial chromatography. The reaction with (S)-alanine methylester demonstrates that the insertion reaction is generally applicable to incorporation of different amino acids into 3,3-disubstituted enollactones. E-enamino ester (**4.06**) was also

of value in assessing the optical purity of the products of the insertion reaction (discussed later in *Section 4.5.3*).

SCHEME 4.07

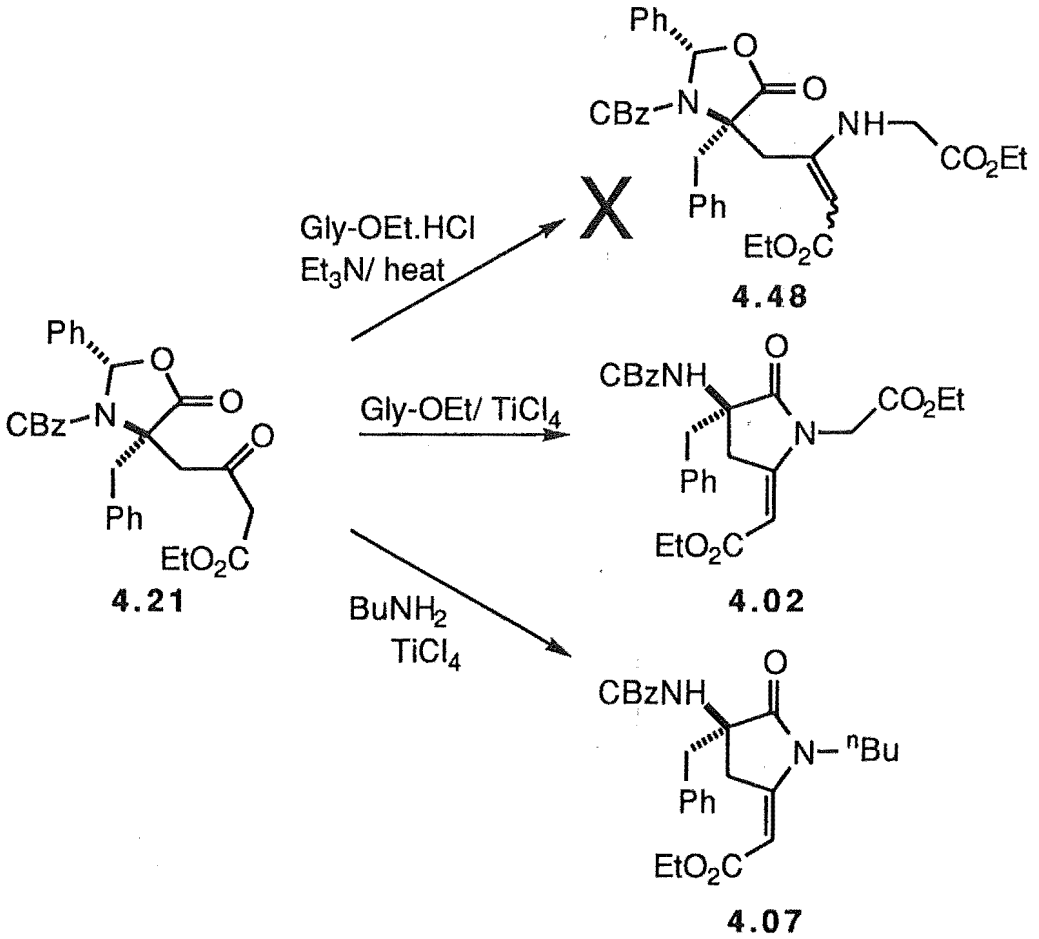


SECTION 4.4.3

SYNTHESIS OF TARGET PROTIO ENAMINO ESTERS FROM β-KETO ESTER

In contrast to the simpler systems described in Chapter 3 (*Sections 3.2-3.3*), the corresponding enamine (**4.48**, SCHEME 4.08) or imine was not detected, by ^1H NMR spectroscopy, following reflux (2-18h) of a 1, 2-dichloroethane or benzene solution of β-keto ester (**4.21**) with glycine ethylester hydrochloride (2-20 equivalent) and triethylamine (2-20 equivalent).

SCHEME 4.08



$TiCl_4$ has been used as a Lewis acid catalyst in cases when formation of enamines has proven difficult[4.12]. $TiCl_4$ also removes H_2O formed in the reaction as TiO_2. Therefore, $TiCl_4$ (0.5 equivalent) was added to β-keto ester (**4.21**) and butylamine (4

equivalent) in toluene, at 0 °C, and after warming to 20 °C the solution was refluxed for 18h. The residue was purified by preparative tlc on silica to give E-enamino ester (**4.07**) in 6% yield (SCHEME 4.08).

Similarly, E-enamino ester (**4.02**) was prepared via the $TiCl_4$ mediated reaction of β-keto ester (**4.21**) with glycine ethylester (10 equivalent) in ether and toluene (SCHEME 4.08). Purification by preparative tlc on silica gave E-enamino ester (**4.02**) in 12% yield. Although not isolated, the enamines (for example **4.48**, SCHEME 4.08) or corresponding imines are the likely precursors of the enamino esters (**4.02** and **4.07**). The yields of enamino esters (**4.02** and **4.07**) were very low, hence the more viable synthesis of the target enamino esters is by the insertion reaction discussed previously (*Sections 4.4.1-4.4.2*).

SECTION 4.5

CHARACTERIZATION OF TARGET ENAMINO ESTERS

SECTION 4.5.1

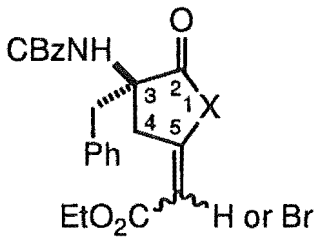
ASSIGNMENT OF E/Z CONFIGURATION TO ENOLLACTONES AND ENAMINO ESTERS

The configuration of the enollactones (**4.42-4.43**) and enamino esters (**4.01-4.07**) was assigned on the basis of ^1H NMR spectroscopy. For the bromo enollactones (**4.42**), the Z isomer (**4.42Z**) was assigned on the basis of a downfield shift of the $(\mathbf{H\text{-}4})_2$ resonance, relative to the E isomer (**4.42E**), which reflects the deshielding effect of CO_2Et (TABLE 4.03). The deshielding effect of CO_2Et was also used to assign E/Z configuration to the enollactones and enamino esters discussed in Chapters 1 and 2-3, respectively. Other differences between the ^1H NMR spectra of E- and Z-bromo enollactones were that the $(\mathbf{H\text{-}4})_2$ protons appeared as an AB quartet in the Z isomer (**4.42Z**) and as a multiplet in the E isomer (**4.42E**), and $OC\mathbf{H_2}CH_3$ appeared as a multiplet in the Z isomer (**4.42Z**) and as a quartet in the E isomer (**4.42E**).

The ylidene carbon, **C-5**, resonance was downfield in the Z isomer (**4.42Z**); δ 159.7, relative to the E isomer (**4.42E**); δ 155.3, which is consistent with the trend observed in the simpler chloro and bromo enollactones (*Section 1.8*, Chapter 1).

The major bromo enamino ester was assigned the Z configuration (**4.01Z**) due to the similarity of its ^{1}H NMR spectrum to that of Z-bromo enollactone (**4.42Z**). For the Z-bromo enamino ester (**4.01Z**), the resonance arising from **(H-4)**$_2$ was at a similar chemical shift to **(H-4)**$_2$ in the Z-bromo enollactone (**4.42Z**) which again reflected the deshielding effect of CO_2Et (TABLE 4.03). Also, the multiplicity of the **(H-4)**$_2$ and OCH_2CH_3 resonances of the major bromo enamino ester (**4.01Z**) were the same as in Z-bromo enollactone (**4.42Z**); namely, an AB quartet and multiplet, respectively.

TABLE 4.03



Compd	E vs Z	δ (H-4)$_a$	δ (H-4)$_b$	J_{AB} (Hz)
4.01Z	Z	3.42	3.92	17.3
4.02	E	3.38	3.89	18.6
4.03	E	3.37	3.79	19.1
4.04	E	3.37	3.82-4.31*	17.6
4.05	E	3.37	3.84	18.6
4.06	E	3.37	3.71	18.6
4.07	E	3.55*	3.80	18.8
4.43	E	3.50	3.82	19.1
4.42Z	Z	3.49	3.80	19.1

No	E vs Z	δ (H-4)$_2$
4.42E	E	3.37 m

* ^{1}H NMR resonance overlapped with other resonances

The remaining protio enollactone (**4.44**) and protio enamino esters (**4.02-4.07**) were assigned the E configuration because the chemical shift of the (**H-4**)$_2$ resonances indicated deshielding by CO_2Et (TABLE 4.03). In the protio enollactone and enamino esters (**4.43, 4.02-4.07**), the (**H-4**)$_2$ resonances were AB quartets at approximately the same chemical shifts as the AB quartet of (**H-4**)$_2$ in the Z-bromo enollactone (**4.42Z**) and Z-bromo enamino ester (**4.01Z**), which have the same relative configuration (TABLE 4.03). Also, for the simpler succinimide-based protio enollactones and acylated enamino esters (*Section 2.3.2*, Chapter 2) the E isomer was invariably favoured.

SECTION 4.5.2

TRENDS IN THE ^{1}H NMR, ^{13}C NMR AND HIGH RESOLUTION MASS SPECTRA OF ENOLLACTONES AND ENAMINO ESTERS

TABLE 4.04 lists characteristic ^{1}H and ^{13}C NMR resonances of enollactones (**4.42-4.43**) and enamino esters (**4.01-4.07**). Key ^{13}C NMR resonances of enamino ester (**4.02**) were able to be assigned following an NMR proton-carbon heteronuclear correlation experiment, and by analogy, characteristic resonances in the ^{13}C NMR spectra of enollactones (**4.42-4.43**) and enamino esters (**4.01, 4.03, 4.06-4.07**) were also assigned. Thus, the range of resonances observed for **C-4**, CH_2Ph, **C-3**, OCH_2Ph, =**C**H and N**C**H$_2$ (where applicable) was δ 36.9-40.3, 42.2-42.5, 59.1-60.4, 67.1-67.7, 92.9-98.7 and 41.4-44.9, respectively (TABLE 4.04).

In the ^{1}H NMR spectra of enollactones (**4.42-4.43**) and enamino esters (**4.01-4.07**) the C3C**H$_2$**Ph and (**H-4**)$_2$ resonances were distinguishable on the basis of the upfield position of C3-C**H$_2$**Ph relative to (**H-4**)$_2$. Also, the geminal coupling constants of the C3C**H$_2$**Ph AB quartets; J=13.0-13.2Hz, was considerably smaller than the geminal coupling constants of the (**H-4**)$_2$ AB quartets; J=17.3-19.1Hz (TABLES 4.02 and 4.03).

For enamino ester (**4.02**) the ^{1}H - ^{13}C NMR correlation experiment showed that the ^{1}H NMR resonance at δ 4.99 was coupled to the ^{13}C NMR resonance at δ 92.9 characteristic of =**C**H, whereas the ^{1}H NMR resonance at δ 5.27 did not show coupling to any ^{13}C NMR resonances. Hence the resonances at δ 4.99 and δ 5.27 were assigned to

TABLE 4.04: Characteristic ^{1}H and ^{13}C NMR Resonances of Enamino Esters and Enollactones

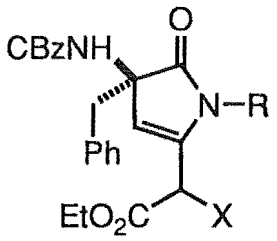
	δ ^{13}C NMR						δ ^{1}H NMR				
No.	**C-4**	NCH_2	$C4CH_2$	**C-3**	OCH_2Ph	=**C**H	=C**H** (s)	N**H** (s)	$C4CH_2Ph$	NCH_2	OCH_2Ph
4.01Z	40.3	44.9	42.5	59.1	67.2	98.7	5.08*	5.29	3.04/3.09 AB_q J=13.2Hz	4.76/4.83 AB_q J=18.4Hz	5.08 m*
4.02	36.9	41.9	42.5	59.3	67.1	92.9	4.99	5.27	3.05 m	4.09/4.43 AB_q J=17.6Hz	5.01/5.10 AB_q J=11.8Hz
4.03	36.9	41.4	42.2	59.1 or 59.8	67.7	92.9	5.11	5.42	2.97/3.11 AB_q J=13.2Hz	3.65/4.67 AB_q J=17.1Hz	5.02 s
4.04	^{13}C NMR spectrum was not recorded						5.29	5.38	3.05 m	3.82-4.31 m*	5.08 m
4.05	^{13}C NMR spectrum was not recorded						5.06	5.38	3.01/3.07 AB_q J=13.2Hz	4.23/4.36 AB_q J=17.5Hz	5.03/5.07 AB_q J=12.2Hz
4.06	37.0	N/A	42.5	59.1 or 59.7	67.1	93.7	5.01*	5.27	2.98/3.07 AB_q J=13.2Hz	N/A	5.01 m*
4.07	^{13}C NMR spectrum was not recorded						4.98	5.27	2.98/3.03 AB_q J=13.0Hz	3.35 m*	5.05/5.08 AB_q J=12.0Hz
4.42E	40.2	N/A	42.5	59.9	67.7	94.6	N/A	5.40	3.03/3.15 AB_q J=13.2Hz	N/A	5.11 m
4.42Z	39.1	N/A	42.5	60.4	67.7	90.7	N/A	5.40	2.98/3.14 AB_q J=13.2Hz	N/A	5.09 m
4.43	37.3	N/A	42.5	59.5	67.7	97.9	5.34	5.46	2.99/3.14 AB_q J=13.2Hz	N/A	5.10 m

* ^{1}H NMR resonance overlapped with other resonances

=CH and NH, respectively. From this result, NH was assigned as downfield relative to =CH for all protio enollactones and enamino esters (**4.43**, **4.02-4.07**).

The mass spectra of the enamino esters, like that of oxazolidinones (**4.11**, **4.14-4.15**, **4.18-4.19**, **4.21-4.22**, **4.25-4.26**, **4.30**, **4.32**, *Section 4.2.4*), characteristically showed a large signal, often the base peak, at M-91 corresponding to M-CH_2Ph.

The endocyclic isomer (**4.49**) was discounted as the product of the insertion reaction between bromo enollactone (**4.42**) and glycine ethylester because ^1H NMR resonances characteristic of the olefinic proton and CHBr were not observed. Instead resonances were observed at δ 3.42 and 3.92, consistent with $(H\text{-}4)_a$ and $(H\text{-}4)_b$ of the enamino ester (**4.01**). The ^1H and ^{13}C NMR spectra of (**4.02-4.07**) showed the same characteristic resonances as those of the bromo enamino ester (**4.01**), hence, by analogy, (**4.02-4.07**) were assigned as enamino esters rather than the corresponding endocyclic isomers (**4.50**). Further, enamino ester (**4.02**) was prepared via the insertion reaction and the enamine route, which is unlikely to give rise to the endocyclic isomer.



4.49 X = Br R = CH_2CO_2Et
4.50 X = H

SECTION 4.5.3

OPTICAL ACTIVITY OF ENAMINO ESTERS

It was expected that the sequence of reactions (SCHEMES 4.01, 4.03-5) leading to the enamino esters (**4.01-4.07**) would not cause racemization at the chiral centre(s). The $(\alpha)_D^{20}$ values of the enamino esters (**4.01-4.07**), enollactones (**4.42-4.43**) and oxazolidinones (SCHEMES 4.01-4.04) showed the compounds to be optically active.

In the ^{13}C NMR spectrum of enamino ester (**4.06**), derived from enollactone (**4.43**) and (S)-alanine methyl ester hydrochloride, less than 5% of another diastereoisomer was

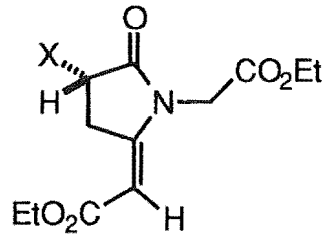
observed. Therefore, it is very unlikely that racemization had occurred at C-3 in enamino ester (**4.06**) or in its precursors. By analogy, the other enamino esters (**4.01-4.05** and **3.16**, Chapter 3) prepared via the same general method are assumed to be optically pure. In the future we expect to confirm the stereochemistry and configuration of the enamino esters (**4.01-4.07**) by single crystal X-ray structure analysis.

SECTION 4.6

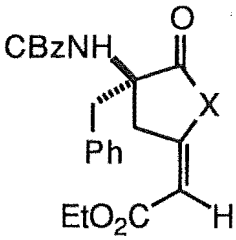
PRELIMINARY TESTING OF ENAMINO ESTERS AND ENOLLACTONES

A colorimetric-based assay was used to measure the inhibition of α-chymotrypsin by enamino esters and enollactones (**2.71**, **3.04**, **4.01-4.03**, **4.06**, **4.42-4.43**). The % inhibition for solutions of enamino esters and enollactones was measured, for a fixed time interval, relative to a control containing α-chymotrypsin and substrate (namely N-succinyl alanine 4-nitroanilide). The results of the assay are summarized in TABLE 4.05.

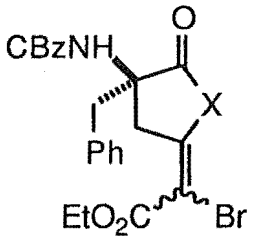
TABLE 4.05



2.71,3.04



4.02-4.03,4.06,4.43



4.01,4.42

Compd	X	concn* (mmol/L)	% I	concn▼ (mmol/L)	% I
2.71	H	7.8×10^{-4}	0	7.8×10^{-1}	5
3.04	CBzNH	4.9×10^{-4}	0	4.9×10^{-1}	25
4.01	NCH_2CO_2Et	3.5×10^{-4}	20	3.5×10^{-1}	40
4.02	NCH_2CO_2Et	4.0×10^{-4}	0	4.0×10^{-1}	40
4.03	$NCH_2CONHCH_2CO_2Et$	3.6×10^{-4}	5	3.6×10^{-1}	40
4.06	(S)-$NCH(Me)CO_2Et$	3.9×10^{-4}	0	3.9×10^{-1}	50
4.42E	O	4.5×10^{-4}	15	4.5×10^{-1}	40
4.42Z	O	4.5×10^{-4}	5	4.5×10^{-1}	35
4.43	O	4.9×10^{-4}	10	4.9×10^{-1}	25
%I = % Inhibition		* all 2×10^{-4}mg/mL		▼ all 2×10^{-1}mg/mL	

Bromo enamino ester (**4.01**), E-bromo enollactone (**4.42E**) and protio enollactone (**4.43**) showed significant inhibition of α-chymotrypsin at concentrations of $2x10^{-4}$ mg/mL.

The results of the α-chymotrypsin assay (TABLE 4.05) for $2x10^{-1}$ mg/mL solutions of enamino esters and enollactones (**2.71**, **3.04**, **4.02**, **4.03**, **4.06** and **4.43**), designed as alternate substrate inhibitors of chymotrypsin, show some interesting trends.

Enamino ester (**2.71**) gave negligible inhibition whereas (**3.04**) gave 25% inhibition which indicates that extension of the peptide chain in the N direction is a viable strategy for optimizing interaction with the target enzyme and thereby increasing inhibition. Similarly, enamino esters extended in the C direction by 1 or 2 amino acid residues; (**4.02**, **4.03** and **4.06**), were more effective inhibitors than the enollactone (**4.43**). In this study attempts were not made to incorporate the potential inhibitors into the optimum oligopeptide.

The greater degree of inhibition exhibited by phenylalanine analogues (**4.02**, **4.03** and **4.06**) compared with enamino esters (**2.71** and **3.04**) illustrates the importance of the aromatic residue at S_1 for chymotrypsin inhibition.

The alanine-derived enamino ester (**4.06**) exhibited a greater degree of inhibition than the corresponding glycine and glyclglycine derived enamino esters (**4.02** and **4.03**, respectively) which indicates that inhibition can be enhanced by incorporation of the inhibitor into a peptide which has optimum interactions with the target enzyme.

Bromo enamino ester and enollactones (**4.01**, **4.42E** and **4.42Z**) were designed as mechanism-based inactivators of α-chymotrypsin and at concentrations of $2x10^{-1}$ mg/mL showed 35-40% inhibition. The E-bromo enollactone (**4.42E**) was a marginally better inhibitor than Z-bromo enollactone. Unlike the protio enamino esters (**4.01**, **4.43**) there was little difference between the bromo enamino esters (**4.01**) and bromo enollactones (**4.42E** and **4.42Z**) with respect to inhibition.

SECTION 4.7

CONCLUSION AND FUTURE WORK

A new general route to enamino esters, involving reaction of enollactones and amines, has been developed and used to synthesize a new class of potential mechanism-based inactivators and alternate substrate inhibitors of chymotrypsin. The potential inhibitors are optically pure, having the same configuration as natural enzyme peptide substrates. The inhibitor is incorporated into a small peptide, which may be varied, and contains a CH_2Ph residue for recognition by chymotrypsin. The potential mechanism-based inactivators contain a latent reactive group which was incorporated via a new reaction involving halo enol-lactonization of keto-acid phosphoranes.

Another route to enamino esters by reaction of β-keto esters with amines was also developed.

The two syntheses of enamino esters are versatile and in the future will be adapted to incorporate the inhibitor into different peptides so that the maximum enzyme-inhibitor interaction is achieved. Also, different recognition groups will be used so that other protease enzymes are targeted.

Use will be made of Me or H, instead of CO_2Et, as the ylidene substituent, to obtain systems which more closely resemble the natural substrate. Further, methods for removal of the CBz group and subsequent addition of amino acid residues to the N terminus will be investigated. A preliminary result showed that the CBz group was removed from enamino ester (**4.05**) upon refluxing in 1, 2-dichloroethane for 5h with PTSA (1.6 equivalent).

SECTION 4.8

CHAPTER 4 REFERENCES

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CHAPTER 5

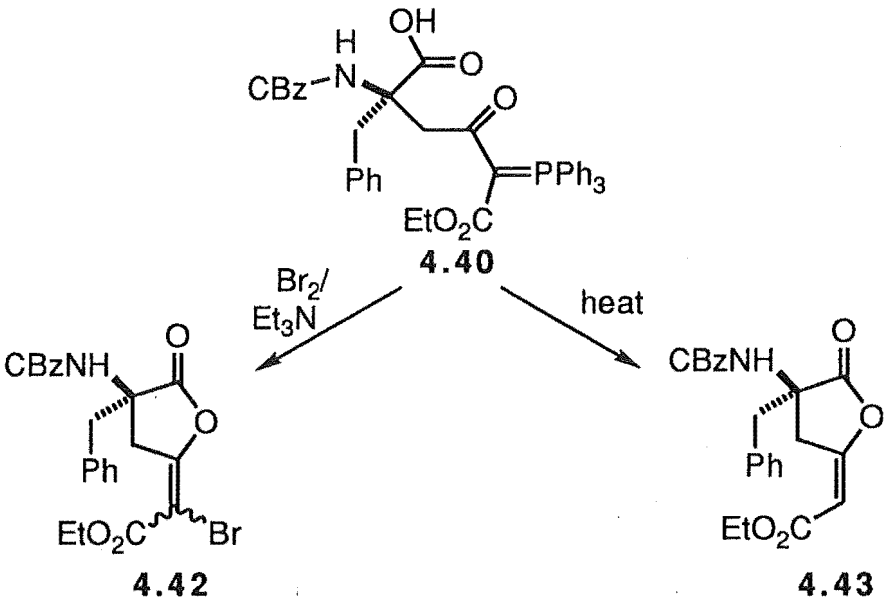
NMR SPECTROSCOPY AND MASS SPECTROMETRY OF KETO ACID AND KETO ESTER PHOSPHORANES

SECTION 5.1

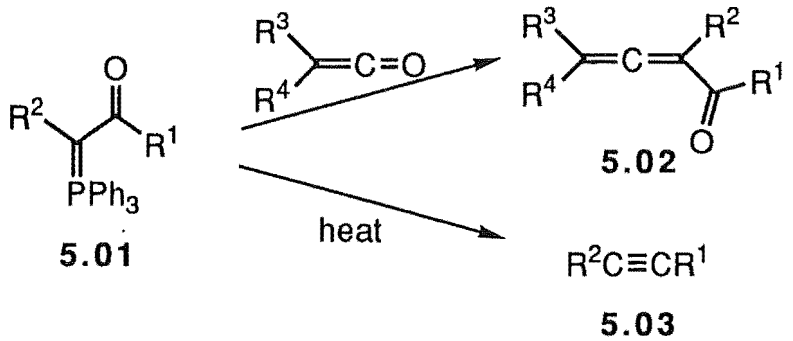
INTRODUCTION

The work presented in Chapter 4 has shown benzyl oxycarbonyl (CBz) keto acid phosphorane (**4.40**) to be an important synthetic intermediate to bromo enollactones (**4.42**) and protio enollactones (for example **4.43**) (SCHEME 5.01). In Chapter 1 extensive use was made of keto acid phosphoranes as synthetic intermediates to bromo and chloro enollactones (See Chapter 1 for more detail). Keto acid phosphoranes are also synthetic intermediates to allenes[5.01] (for example **5.02**) and acetylenes[5.02] (for example **5.03**) (SCHEME 5.02).

SCHEME 5.01



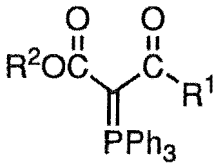
SCHEME 5.02

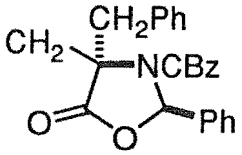
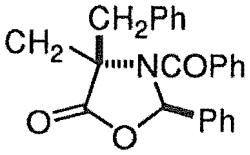
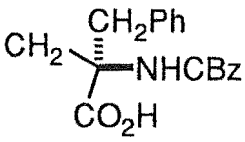
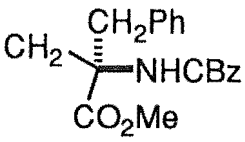


Another application of keto acid phosphoranes is in the study of keto acid hydrogen-bonding patterns[5.03].

A detailed study of the NMR spectroscopy and, in particular, the high resolution mass spectrometry of keto acid and keto ester phosphoranes (**4.15**, **4.26**, **4.40-4.41**, TABLE 5.01), introduced in Chapter 4, was undertaken. To provide further examples of this class of compound the ^{t}butyl keto acid and keto ester phosphoranes (**5.04-5.06**, TABLE 5.01) were also prepared and studied.

TABLE 5.01



No	R^1	R^2
4.15		Et
4.26		Et
4.40		Et
4.41		Et
5.04	$(CH_2)_2CO_2CHPh_2$	^{t}Bu
5.05	$(CH_2)_2CO_2H$	^{t}Bu
5.06	$(CH_2)_2CO_2Me$	^{t}Bu

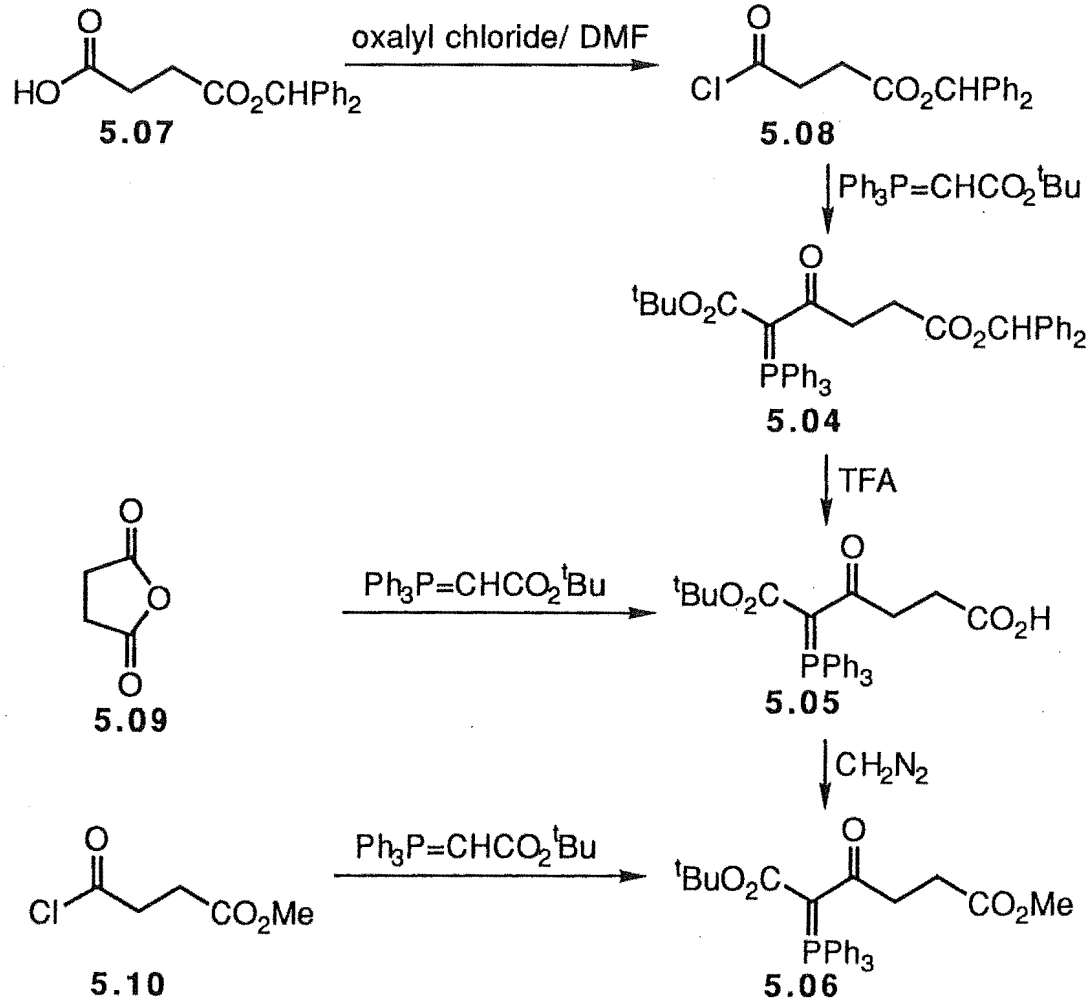
SECTION 5.2

SYNTHESIS OF KETO ACID AND KETO ESTER PHOSPHORANES

Keto acid phosphoranes (**4.15** and **4.26**, TABLE 5.01) were prepared via reaction of the appropriate acid chloride with $Ph_3P{=}CHCO_2Et$ and also by alkylation of the appropriate oxazolidinone with $BrCH_2COC(PPh_3)CO_2Et$. Phosphorane (**4.15**) existed as a 1 : 1 mixture of conformers, by ^1H NMR. Keto ester phosphorane (**4.40**, TABLE 5.01) was prepared by selective hydrolysis of oxazolidinone (**4.15**) and, on treatment with CH_2N_2, gave the methyl ester (**4.41**, TABLE 5.01). More detail on the synthesis of phosphoranes (**4.15**, **4.26** and **4.40-4.41**) is given in Chapter 4 (*Sections 4.2* and *4.4.1*, respectively).

The synthesis of keto acid and keto ester phosphoranes (**5.04-5.06**) is summarized in SCHEME 5.03.

SCHEME 5.03



Benzhydryl phosphorane (**5.04**) was prepared, in 91% yield after radial chromatography, via reaction of $Ph_3P{=}CHCO_2{}^tBu$ (2 equivalent) with $Ph_2CHO_2C(CH_2)_2COCl$ (**5.08**) in benzene. Benzhydryl ester phosphorane (**5.04**) was also prepared, in the reduced yield of 27%, via the above method with $Ph_3P{=}CHCO_2{}^tBu$ (1 equivalent) and $^iPr_2NEt$ (1 equivalent).

Deprotection of benzhydryl phosphorane (**5.04**), with TFA (trifluoroacetic acid), quantitatively gave the keto acid phosphorane (**5.05**). Keto acid phosphorane (**5.05**) was also prepared from the reaction of succinic anhydride (**5.09**) with $Ph_3P{=}CHCO_2{}^tBu$, in CH_2Cl_2. The crude residue contained, by ^1H NMR spectroscopy, 46% keto acid phosphorane (**5.05**) : 27% succinic anhydride (**5.09**) : 27% $Ph_3P{=}CHCO_2{}^tBu$. Without further purification the residue was dissolved in THF and treated with an excess of CH_2N_2 to give the methyl ester phosphorane (**5.06**), which was isolated in a yield of 41% following radial chromatography. Methyl ester phosphorane (**5.06**) was also prepared, in 51% yield, from the reaction of $MeO_2C(CH_2)_2COCl$ (**5.10**) with $Ph_3P{=}CHCO_2{}^tBu$ (2 equivalent) in benzene.

SECTION 5.3

^{13}C and ^{31}P NMR SPECTROSCOPY OF KETO ACID AND KETO ESTER PHOSPHORANES

Traditionally, keto ester and keto acid phosphoranes have been identified on the basis of ^1H, ^{13}C and ^{31}P NMR spectroscopy[5.04]. The ^{13}C and ^{31}P NMR data of keto acid and keto ester phosphoranes (**4.15, 4.26, 4.40-4.41, 5.04-5.06**) were typical of that of previously studied phosphorous compounds[5.04]. Characteristic ^{13}C NMR resonances of keto acid and keto ester phosphoranes (**4.15, 4.26, 4.40-4.41, 5.04-5.06**), assigned by comparison of the chemical shifts and ^{13}C - ^{31}P coupling constants with those of reported phosphorous compounds[5.04], are shown in TABLE 5.02. The broad band decoupled ^{31}P NMR spectra showed that the phosphorous atom in keto acid and keto ester phosphoranes (**4.15, 4.26, 4.40-4.41, 5.04-5.06**) also resonated in a characteristic chemical shift range; δ 17-19 (downfield relative to 85% H_3PO_4) (TABLE 5.02).

TABLE 5.02: Characteristic Resonances in the ^{13}C and ^{31}P NMR Spectra of Phosphoranes

(4.15, 4.26, 4.40-4.41, 5.04-5.06)

No.	Characteristic ^{13}C NMR resonances δ doublet (J in Hz)								^{31}P δ
	CH_2CO	$C{=}PPh_3$	CO_2R	CH_2CO	*Ipso*	*ortho*	*meta*	*para*	**P**
4.15▼	46.0 (7.6) and 48.0 (7.1)	71.1 (109.3) and 71.2 (110.8)	167.3 (14.1) and 167.5 (14.5)	192.1 (6.0) and 192.2 (5.1)	125.6 (93.7) and 125.9 (93.1)	133.2 (10.1) and 133.2 (9.6)	128.6 (12.6)	131.6 (2.5)	18.1
4.26	45.9 (7.1)	70.8 (110.6)	167.4 (14.4)	192.9 (4.6)	125.3 (93.2)	133.4 (10.0)	128.5 (12.6)	131.7 (2.9)	18.3
4.40	41.5 (6.1)	*	166.6 (13.1)	192.8 (4.0)	124.4 (93.7)	133.1 (10.0)	128.8 (13.1)	132.4 (2.1)	18.6
4.41	45.6 (6.1)	71.8 (110.8)	167.6 (15.1)	193.1 (4.0)	126.3 (93.7)	132.2 (10.0)	127.9 (14.1)	131.6 (3.0)	18.0
5.04	35.2 (7.6)	70.5 (109.5)	167.3 (13.7)	194.6 (4.3)	127.1 (93.6)	133.0 (9.7)	128.4 (12.7)	131.4 (2.9)	17.7
5.05	33.6 (7.3)	73.8 (107.6)	166.6 (12.4)	196.5 (3.7)	125.5 (93.7)	133.0 (10.1)	128.8 (12.5)	132.1 (3.0)	17.3
5.06	35.4 (7.3)	*	167.4 (13.8)	194.9 (4.5)	127.2 (93.1)	133.0 (9.8)	128.5 (12.1)	131.4 (3.0)	17.2

▼ Keto acid phosphorane (**4.15**) existed as a 1 : 1 mixture of conformers (*Section 4.2.1*, Chapter 4) and data is given for both conformers

* Insufficient sample to obtain $C{=}PPh_3$

SECTION 5.4

FAB MASS SPECTROMETRY OF KETO ACID AND KETO ESTER PHOSPHORANES

Fast atom bombardment (FAB) mass spectra of the keto acid and keto ester phosphoranes (**4.15, 4.26, 4.40-4.41, 5.04-5.06**) were also characteristic. TABLE 5.02 lists the relative abundance of positive ions observed in the FAB mass spectra of phosphoranes (**4.15, 4.26, 4.40-4.41, 5.04-5.06**).

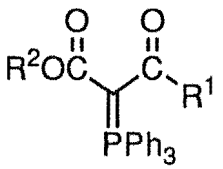
TABLE 5.02 Relative Abundance of Positive Ions in FAB Mass Spectra of the Keto Acid and Keto Ester Phosphoranes (**4.15, 4.26, 4.40-4.41, 5.04-5.06**)

No	*m/z* (relative intensity, %)
4.15	776 (38), 730 (35), 390 (35), 375 (80), 303 (100), 279 (32), 262 (51), 201 (51), 183 (80)[a]
4.26	746 (81), 700 (56), 390 (65), 375 (89), 303 (100), 279 (26), 262 (63), 201 (38), 183 (56), 165 (45)[a]
4.40	688 (45), 642 (20), 612 (21), 508 (31), 390 (41), 375 (71), 349 (25), 303 (100), 279 (39), 262 (53), 225 (20), 201 (38), 183 (65)[a]
4.41	702 (86), 656 (22), 390 (64), 375 (71), 303 (100), 279 (21), 262 (43), 201 (22), 183 (38)[a]
5.04	643 (10), 569 (8), 403 (15), 347 (30), 303 (30), 279 (7), 262 (8), 201 (34), 183 (15), 167 (100), 152 (12)[b]
5.05	477 (41), 403 (54), 377 (58), 347 (45), 321 (99), 303 (82), 279 (29), 262 (24), 201 (21), 183 (58), 152 (100)[b]
5.06	491 (41), 417 (57), 403 (11), 347 (56), 303 (100), 279 (26), 262 (15), 201 (18), 183 (32), 152 (16)[b]
	[a] Spectra run in nitrobenzyl alcohol
	[b] Spectra run in "magic bullet" (*ie* 1 dithioerythritol : 5 dithiothreitol)

All spectra showed the protonated molecular ion $(MH)^+$ and a common fragmentation pattern. Fragment ions occurred at $(MH-74)^+$ for ^{t}butyl phosphoranes (**5.04-5.06**), which corresponds to loss ^{t}butanol, and at $(MH-46)^+$ for ethyl phosphoranes (**4.15, 4.26, 4.40-4.41**) which corresponds to loss of ethanol. Loss of R^1 resulted in fragment ions at m/z 403 for ^{t}butyl phosphoranes (**5.04-5.06**) and at m/z 375 for ethyl phosphoranes (**4.15, 4.26, 4.40-4.41**). Characteristic fragment ions were also observed at m/z 303 ($C_{20}H_{16}OP$), 279 ($C_{18}H_{16}OP$), 262 ($C_{18}H_{15}P$) and 201 ($C_{12}H_{10}OP$) for ethyl and ^{t}butyl phosphoranes (**4.15, 4.26, 4.40-4.41, 5.04-5.06**).

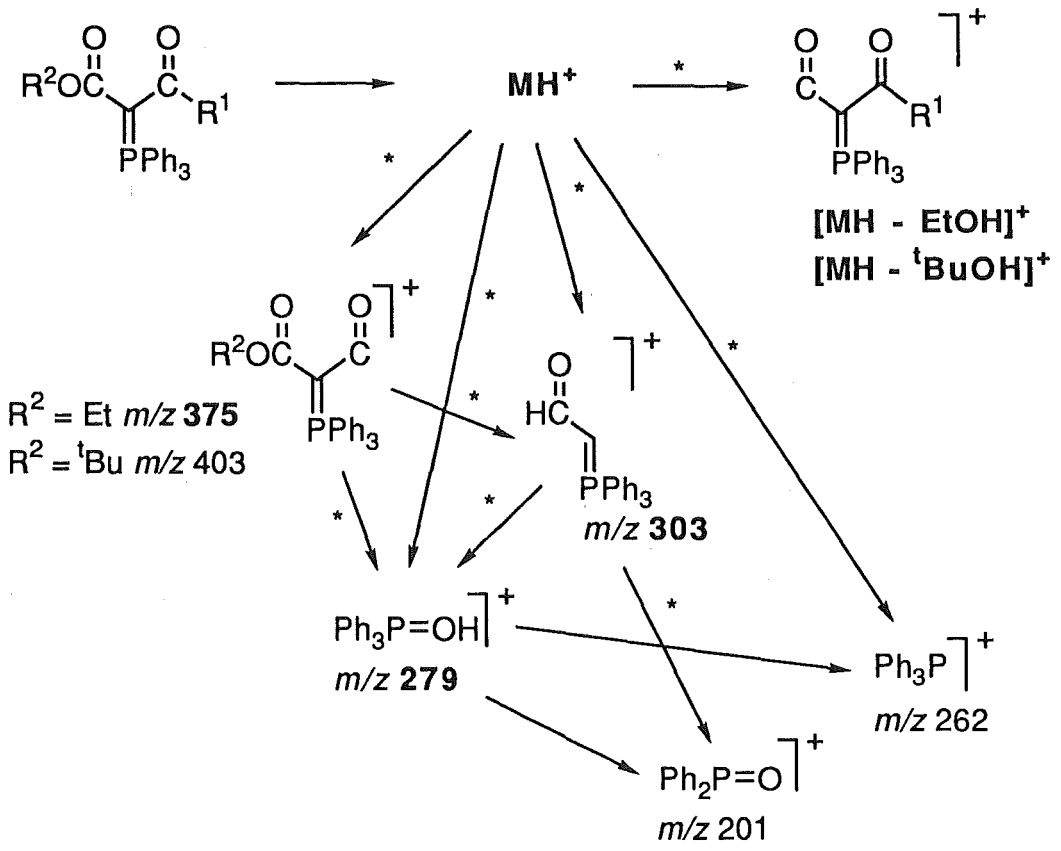
FAB mass spectra of seven other keto acid and keto ester phosphoranes[5.05] (**5.11-5.17**, TABLE 5.03), each with R^2=Et also showed the protonated molecular ion $(MH)^+$ and the same fragmentation pattern as phosphoranes (**4.15, 4.26, 4.40-4.41, 5.04-5.06**); namely, signals at m/z 375 (loss of R^1), 303, 279, 262, 201 and $(MH-46)^+$. The fragmentation pattern of the phosphoranes (**4.15, 4.26, 4.40-4.41, 5.04-5.06, 5.11-5.17**), summarized in SCHEME 5.04, was supported by the results of metastable decompositions for phosphoranes (**5.12, 5.15-5.16**) and high resolution results.

TABLE 5.03



No	R^1	R^2
5.11	$(CH_2)_2CO_2H$	Et
5.12	$(CH_2)_3CO_2H$	Et
5.13	$(CH_2)_4CO_2H$	Et
5.14	$CHBr(CH_2)_3CO_2H$	Et
5.15	CH_2CO_2Me	Et
5.16	$(CH_2)_2CO_2CHPh_2$	Et
5.17	$(CH_2)_4CO_2Me$	Et

SCHEME 5.04: FAB Positive Ion Fragmentation Pathway for Keto Acid and Keto Ester Phosphoranes (**4.15, 4.26, 4.40-4.41, 5.04-5.06**)

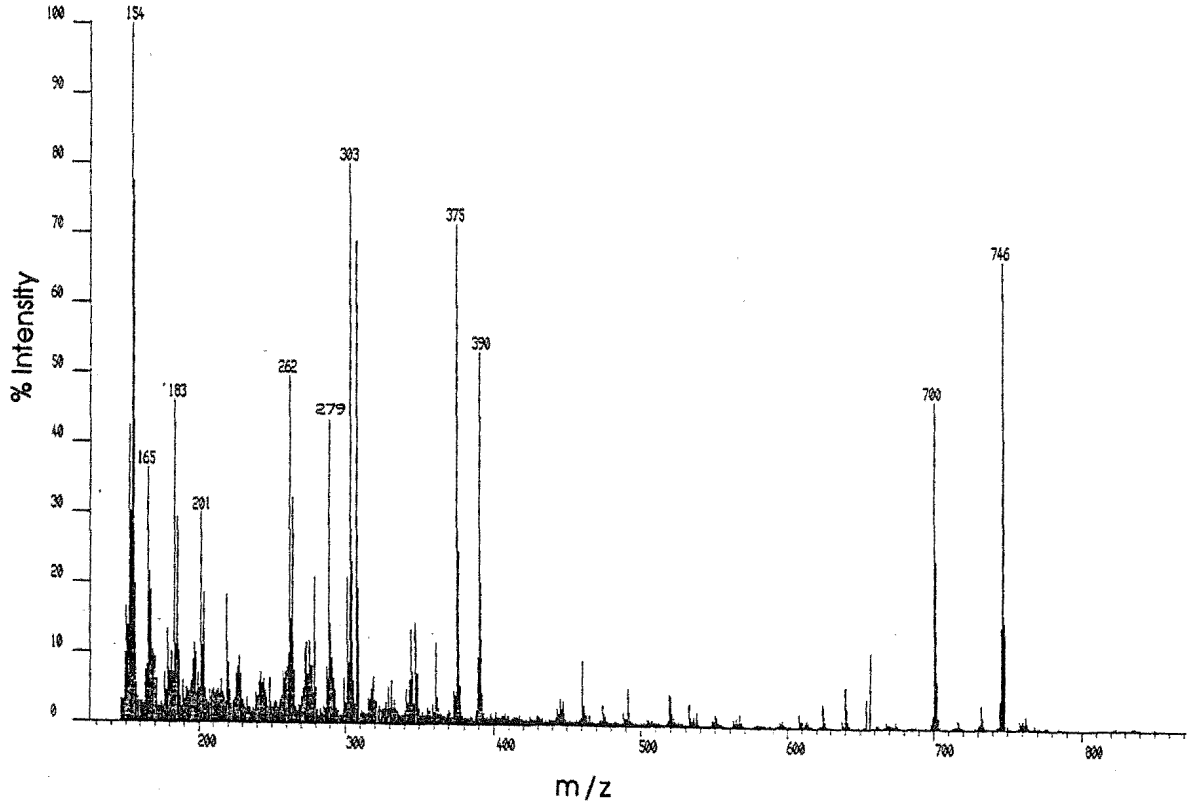


Bold lettering designates high resolution result

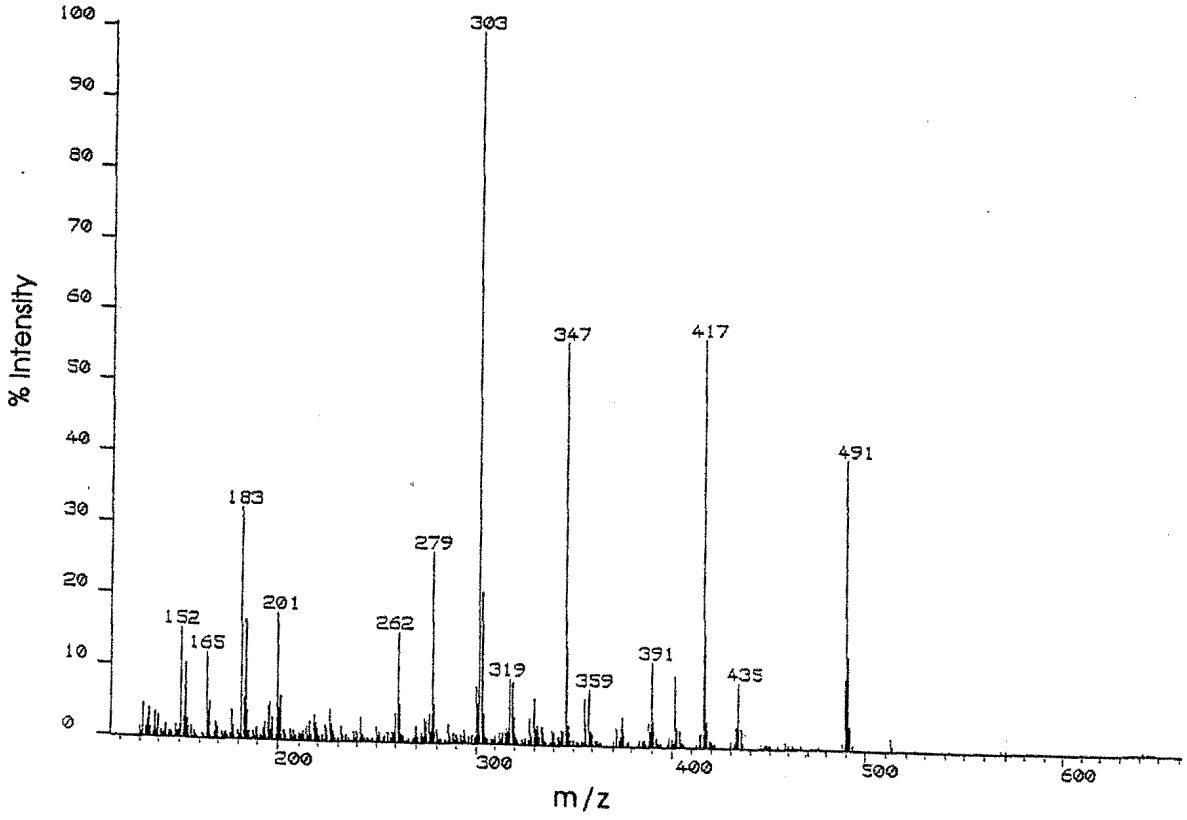
* Verified by metastable transitions for phosphoranes (**5.12, 5.15** and **5.16**)

Representative mass spectra; of phosphoranes (**4.26**) and (**5.06**), are shown; Spectrum 5.01 and Spectrum 5.02, respectively.

Spectrum 5.01

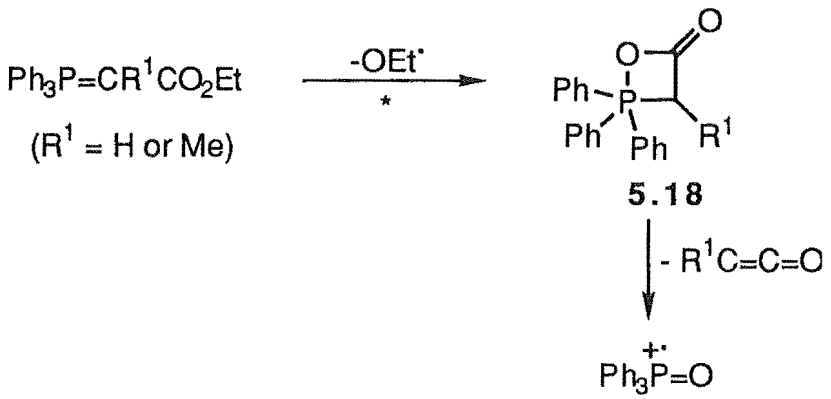


Spectrum 5.02



In the literature, mass spectral data of phosphoranes is limited to positive and negative ion electron impact (EI) mass spectrometry of simple triphenyl phosphoranes[5.06-5.07]. It is reported[5.07] that O alkyl cleavage to give the species (**5.18**), followed by loss of $R^1C{=}C{=}O$ gives rise to the ion observed at m/z 278 in the EI spectra of simple triphenylphosphoranes (SCHEME 5.05). The formation of (**5.18**) was substantiated by the observation of a metastable peak. The protonated version of the fragment ion m/z 278 is observed in the FAB mass spectra of keto acid and keto ester phosphoranes (**4.15, 4.26, 4.41, 5.04-5.06**) at m/z 279, and it is probably formed from the protonated molecular ion $(MH)^+$ via the same mechanism.

SCHEME 5.05



* Verified by a metastable transition

SECTION 5.5

CONCLUSION

Keto acid and keto ester phosphoranes are important as synthetic intermediates to enollactones, allenes and acetylenes and also in the study of keto acid hydrogen-bonding patterns. The resonances observed in the ^{31}P and ^{13}C NMR spectra of keto acid and keto ester phosphoranes (**4.15, 4.26, 4.40-4.41, 5.04-5.06**) occurred at characteristic chemical shifts and the ^{13}C - ^{31}P coupling constants in the ^{13}C NMR spectra were also diagnostic. The FAB mass spectra of phosphoranes (**4.15, 4.26, 4.40-4.41, 5.04-5.06**) showed the protonated molecular ion and a common fragmentation pattern; fragment ions were observed corresponding to $(MH-46)^+$ and at m/z 375, 303, 279, 262, 201 and for the ^{t}butyl phosphoranes (**5.04-5.06**), and corresponding to $(MH-76)^+$ and at m/z 403, 303, 279, 262, 201 for ethyl phosphoranes (**4.15, 4.26, 4.40-4.41**). Hence, on the basis of the fragmentation pattern observed in FAB mass spectrometry, it is possible to identify keto acid and keto ester phosphoranes.

SECTION 5.6

CHAPTER 5 REFERENCES

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EXPERIMENTAL

GENERAL METHODS

Melting points less than 200 °C were measured using a Reichert micro heating stage and are uncorrected. Melting points greater than 200 °C were measured using an Electrothermal melting point Apparatus and are uncorrected.

Infrared spectra were measured using a Pye Unicam SP3-300 (denoted in the text as IR) or Perkin Elmer 1600 FTIR (denoted in the text as FTIR) Spectrophotometer, and were referenced on the polystyrene 1603cm^{-1} absorbance.

Nuclear Magnetic Resonance (NMR) spectra were obtained on a Varian XL 300 Spectrometer operating at 300 MHz for ^{1}H NMR, 75.5 MHz for ^{13}C NMR and 121.5 MHz for ^{31}P NMR. ^{1}H NMR spectra run in $CDCl_3$ used a trimethylsilane internal standard, and ^{31}P NMR used an external phosphoric acid reference. ^{1}H NMR is reported as ppm (multiplicity, coupling constant(s) in Hz, assignment).

Mass spectra were obtained using a Kratos MS80RFA magnetic sector double focusing mass spectrometer.

ORD spectra were recorded using a JASCO J-20C Recording Spectropolarimeter and are reported as ± ° (concentration in g/100mL; solvent).

Preparative Chromatography was carried out using a Chromatotron (Harrison Research Inc.); a centrifugally accelerated radial thin layer chromatograph, using glass plates coated with silica gel (P.F. 254 60) of 1mm, 2mm or 4mm thickness. Visualisation of non-coloured compounds was achieved using an ultraviolet lamp.

Solvents and chemicals, where necessary, were purified by standard techniques[E.01].

All reactions were carried out under a dry N_2 atmosphere. Following reaction the solvent was evaporated under reduced pressure (Büchi Rotary Evaporator, 20mm), unless otherwise stated.

SECTION E.1

CHAPTER 1 EXPERIMENTAL

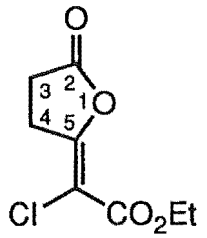
SECTION E.1.1

PREPARATION OF CHLORO AND BROMO ENOLLACTONES (**1.11-1.15, 1.17**)

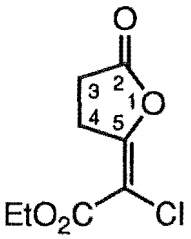
General Method for the Preparation of Chloro Enollactones (**1.11-1.13**):

A solution of the keto acid phosphorane[E.02] (**1.06-1.08**) (stated amount, 1equiv) in CH_2Cl_2, was cooled to -78 °C and SO_2Cl_2 (1.5equiv), followed by triethylamine (1.5equiv) were added. The solution was stirred at -78 °C for 30min and was then allowed to warm to 20 °C. The solvent was evaporated and the residue was purified by radial chromatography on a 2mm silica gel chromatotron plate, eluting with the stated solvent system.

(E)- and (Z)-5-chloroethoxycarbonylmethylidene-2-tetrahydrofuranone (**1.11E** and **1.11Z**):



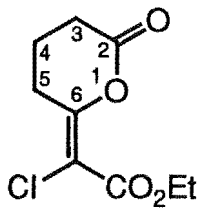
1.11E



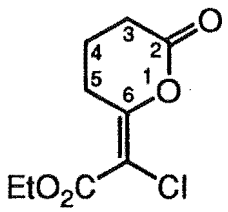
1.11Z

General method with phosphorane (**1.06**) (370mg, 0.83mmol), SO_2Cl_2 (100μL, 1.24mmol) and triethylamine (163μL, 1.24mmol) in CH_2Cl_2 (20mL). Elution with 55% petroleum ether/45% ethyl acetate gave an inseparable mixture of E- and Z-chloro enollactones (**1.11E** and **1.11Z**, respectively) (86% E : 14% Z, by ^1H NMR) as an oil (156mg, 92%): IR (film) 1840, 1720 and 1650cm^{-1}; ^1H NMR ($CDCl_3$) E isomer (**1.11E**) from mixture δ 1.35 (t, J=7.2Hz, OCH_2CH_3), 2.78 (m, (**H-3**)$_2$), 3.14 (m, (**H-4**)$_2$), 4.32 (q, J=7.2Hz, OCH_2CH_3), Z isomer (**1.11Z**) from mixture δ 1.36 (t, J=7.1Hz, OCH_2CH_3), 2.83 (m, (**H-3**)$_2$), 3.43 (m, (**H-4**)$_2$), 4.30 (q, J=7.1Hz, OCH_2CH_3); ^{13}C NMR ($CDCl_3$) E isomer (**1.11E**) from mixture δ 14.01, 25.48, 27.07, 61.96, 104.14, 158.50, 160.88, 173.27, Z isomer (**1.11Z**) from mixture δ 14.03, 26.48, 27.32, 61.88, 101.40, 161.58, 162.87, 172.32. HRMS (M) Found 204.0190 (Calcd for $C_8H_9{}^{35}ClO_4$ 204.0190). Anal. Calcd for $C_8H_9ClO_4$: C 46.96; H 4.43; Cl 17.33. Found: C 46.36; H 4.32; Cl 17.30.

(E)- and (Z)-6-chloroethoxycarbonylmethylidene-2-tetrahydropyrone (**1.12E** and **1.12Z**):



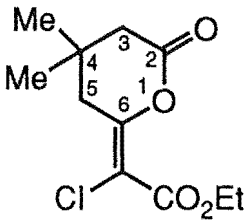
1.12E



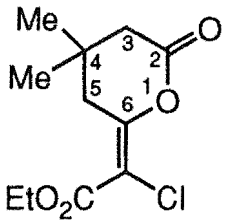
1.12Z

General method with phosphorane (**1.07**) (100mg, 0.22mmol), SO_2Cl_2 (26μL, 0.32mmol) and triethylamine (43μL, 0.32mmol) in CH_2Cl_2 (4mL). Elution with 85% petroleum ether/15% ethyl acetate gave an inseparable mixture of E- and Z-chloro enollactones (**1.12E** and **1.12Z**, respectively) (96% E : 4% Z, by ^1H NMR) as an oil (35mg, 73%): IR (nujol) 1790, 1720 and 1620cm^{-1}; ^1H NMR ($CDCl_3$) E isomer (**1.12E**) from mixture δ 1.35 (t, J=7.1Hz, OCH_2CH_3), 2.00 (quin, J=6.7Hz, (**H-4**)$_2$), 2.65 (t, J=6.7Hz, (**H-3**)$_2$), 2.83 (t, J=6.7Hz, (**H-5**)$_2$), 4.31 (q, J=7.1Hz, OCH_2CH_3), Z isomer (**1.12Z**) from mixture δ 1.35 (t, J=7.2Hz, OCH_2CH_3), 1.97 (quin, J=6.6Hz, (**H-4**)$_2$), 2.72 (t, J=6.6Hz, (**H-3**)$_2$), 3.20 (t, J=6.6Hz, (**H-5**)$_2$), 4.28 (q, J=7.2Hz, OCH_2CH_3); ^{13}C NMR ($CDCl_3$) E isomer (**1.12E**) from mixture δ 14.13, 17.15, 25.51, 30.08, 62.20, 108.57, 155.42, 161.60, 165.39; HRMS (M) Found 218.0348 (Calcd for $C_9H_{11}{}^{35}ClO_4$ 218.0346).

(E)- and (Z)-6-chloroethoxycarbonylmethylidene-4,4-dimethyl-2-tetrahydropyrone (**1.13E** and **1.13Z**):



1.13E



1.13Z

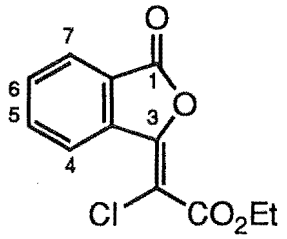
General method with phosphorane (**1.08**) (100mg, 0.20mmol), SO_2Cl_2 (25μL, 0.31mmol) and triethylamine (40μL, 0.31mmol) in CH_2Cl_2 (4mL). Elution with 65% petroleum ether/35% ethyl acetate gave an inseparable mixture of E- and Z-chloro enollactones (**1.13E** and **1.13Z**, respectively) (88% E : 12% Z, by ^1H NMR) as an oil (35mg, 70%): ^1H NMR ($CDCl_3$) E isomer (**1.13E**) from mixture δ 1.13 (s, $C(CH_3)_2$), 1.35 (t, J=7.1Hz, OCH_2CH_3), 2.49 (m, (**H-3**)$_2$), 2.66 (m, (**H-5**)$_2$), 4.32 (q, J=7.1Hz, OCH_2CH_3), Z isomer from mixture (**1.13Z**) δ 1.10 (s, $C(CH_3)_2$), 1.36 (t, J=7.1Hz, OCH_2CH_3), 2.54 (m, (**H-3**)$_2$), 3.05 (m, (**H-5**)$_2$), 4.28 (q, J=7.1Hz, OCH_2CH_3); ^{13}C NMR ($CDCl_3$) E isomer (**1.13E**) from mixture δ 14.06, 27.96, 29.74, 38.95, 43.53, 62.12, 108.91,

154.58, 161.56, 165.06; HRMS (M) Found 248.0646 (Calcd for $C_{11}H_{15}{}^{37}ClO_4$ 248.0630), Found 246.0659 (Calcd for $C_{11}H_{15}{}^{35}ClO_4$ 246.0660).

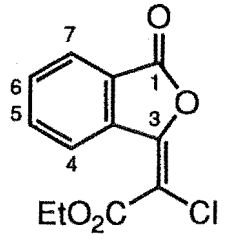
General Method for the Preparation of Phthalic-Based Chloro Enollactones (1.14-1.15):

$Ph_3P{=}CHCO_2Et$[E,03] (1.1equiv) was added to a stirred solution of anhydride (stated amount, 1equiv), in CH_2Cl_2 (8mL), at 0 °C. After 15min SO_2Cl_2 (1.5equiv), followed by triethylamine (1.5equiv), were added and the solution was stirred for 1h at 0 °C. The solvent was evaporated and a ^1H NMR spectrum of the crude mixture allowed estimation of the E/Z isomer ratio. The products were purified by radial chromatography using a 2mm silica gel chromatotron plate, eluting with the stated solvent system.

(E)- and (Z)-3-chloroethoxycarbonylmethylidene phthalide (**1.14E** and **1.14Z**):



1.14E

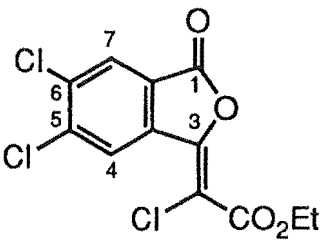


1.14Z

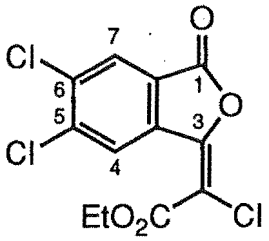
General method with $Ph_3P{=}CHCO_2Et$ (181mg, 0.52mmol), phthalic anhydride (69mg, 0.47mmol), SO_2Cl_2 (56μL, 0.70mmol) and triethylamine (93μL, 0.70mmol) in CH_2Cl_2 (8mL) gave E- and Z- chloro enollactones (**1.14E** and **1.14Z**, respectively) (44% E : 56% Z, by ^1H NMR). Elution with 56% petroleum ether/44% CH_2Cl_2 gave Z-chloro enollactone (**1.14Z**) as a white solid (42mg, 35%) (Another radial chromatographic step, eluting with 75% petroleum ether/25% ethyl acetate, was necessary to remove unreacted phthalic anhydride): mp 114-115 °C (petroleum ether, colourless crystals); IR (KBr) 1800, 1720, 1620 and 1590cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.44 (t, J=7.1Hz, OCH_2CH_3), 4.43 (q, J=7.1Hz, OCH_2CH_3), 7.68 (dt, J=1.0, 7.5Hz, **H-5**), 7.80 (dt, J=1.3, 8.1Hz, **H-6**), 7.99 (td, J=1.0, 7.5Hz, **H-7**), 8.72 (td, J=0.8, 8.1Hz, **H-4**); ^{13}C NMR ($CDCl_3$) δ 14.08, 62.79, 108.07, 125.88, 126.15, 127.22, 132.05, 135.39, 135.71, 152.93, 162.65, 164.44; HRMS (M) Found 254.0151 (Calcd for $C_{12}H_9{}^{37}ClO_4$ 254.0160), Found 252.0187 (Calcd for $C_{12}H_9{}^{35}ClO_4$ 252.0190). Anal. Calcd for $C_{12}H_9ClO_4$: C 57.05; H 3.59; Cl

14.03. Found: C 56.90; H 3.50; Cl 14.52. Further elution gave E-chloro enollactone (**1.14E**) as a white solid (32mg, 27%): mp 137-139 °C (petroleum ether, colourless crystals); IR (KBr) 1790, 1720 and 1630cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, J=7.1Hz, $OCH_2\mathbf{CH_3}$), 4.43 (q, J=7.1Hz, OCH_2CH_3), 7.73 (dt, J=1.0, 7.5Hz, **H-5**), 7.85 (dt, J=1.3, 7.7Hz, **H-6**), 8.04 (td, J=1.0, 7.6Hz, **H-7**), 8.49 (td, J=0.8, 8.0Hz, **H-4**); ^{13}C NMR ($CDCl_3$) δ 14.17, 62.93, 108.31, 125.72, 126.24, 126.65, 132.29, 135.22, 137.46, 149.67, 161.48, 164.77; HRMS (M) Found 254.0190 (Calcd for $C_{12}H_9{}^{37}ClO_4$ 254.0160), Found 252.0186 (Calcd for $C_{12}H_9{}^{35}ClO_4$ 252.0190). Anal. Calcd for $C_{12}H_9ClO_4$: C 57.05; H 3.59; Cl 14.03. Found: C 56.90; H 3.51; Cl 14.32.

*(E)- and (Z)-3-chloroethoxycarbonylmethylidene 5,6-dichlorophthalide (**1.15E** and **1.15Z**):*



1.15E

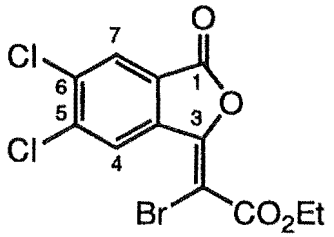


1.15Z

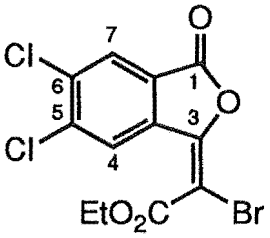
General method with $Ph_3P{=}CHCO_2Et$ (181mg, 0.52mmol), 4,5-dichlorophthalic anhydride (102mg, 0.47mmol), SO_2Cl_2 (56μL, 0.70mmol) and triethylamine (93μL, 0.70mmol) in CH_2Cl_2 (8mL) gave E- and Z-chloro enollactones (**1.15E** and **1.15Z**, respectively) (23% E : 77% Z, by ^{1}H NMR): Elution with 60% CH_2Cl_2/40% petroleum ether gave Z-chloro enollactone (**1.15Z**) as a white solid (109mg, 72%): mp 147-149 °C (petroleum ether, colourless crystals); IR (KBr) 1810, 1720 and 1620cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.44 (t, J=7.1Hz, $OCH_2\mathbf{CH_3}$), 4.44 (q, J=7.1Hz, $O\mathbf{CH_2}CH_3$), 8.04 (s, **H-7**), 8.96 (s, **H-4**); ^{13}C NMR ($CDCl_3$) δ 14.04, 63.17, 109.73, 125.54, 127.11, 129.31, 134.47, 137.21, 140.63, 151.41, 162.26, 162.35; HRMS (M) Found 321.9359 (Calcd for $C_{12}H_7{}^{37}Cl^{35}Cl_2O_4$ 321.9381), Found 319.9418 (Calcd for $C_{12}H_7{}^{35}Cl_3O_4$ 319.9411). Anal. Calcd for $C_{12}H_7Cl_3O_4$: C 44.82; H 2.19; Cl 33.08. Found: C 44.75; H 2.05; Cl 33.30. Further elution gave E-chloro enollactone (**1.15E**) as a white solid (32mg, 21%): mp 171-173 °C (petroleum ether); IR (KBr) 1800, 1720 and 1630cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.42 (t, J=7.1Hz, $OCH_2\mathbf{CH_3}$), 4.43 (q, J=7.1Hz, $O\mathbf{CH_2}CH_3$), 8.09 (s, **H-7**), 8.58 (s, **H-4**); ^{13}C NMR ($CDCl_3$) δ 14.11, 63.20, 109.68, 125.05, 127.60, 128.09, 136.28, 137.57, 140.49, 147.80, 160.89, 162.62; HRMS (M) Found 321.9394 (Calcd for $C_{12}H_7{}^{37}Cl^{35}Cl_2O_4$ 321.9381), Found 319.9411 (Calcd for

$C_{12}H_7{}^{35}Cl_3O_4$ 319.9411). Anal. Calcd for $C_{12}H_7Cl_3O_4$: C 44.82; H 2.19; Cl 33.08. Found: C 44.53; H 1.98; Cl 33.68.

Preparation of Ethyl (E)- and (Z)-bromo-(5,6-dichloro-3-oxo-1,3-dihydroisobenzofuran-1-ylidene)acetate (**1.17E** and **1.17Z**):



1.17E



1.17Z

METHOD A: $Ph_3P{=}CBrCO_2Et$[E.04] (538mg, 1.26mmol, 1.1equiv) was added to a stirred solution of 4,5-dichlorophthalic anhydride (**1.16**) (246mg, 1.13mmol, 1equiv), dissolved in CH_2Cl_2 (20mL), at 20 °C. After 5h at 20 °C, the solvent was evaporated and a ^1H NMR spectrum of the crude product revealed an isomer ratio of 20% E : 80% Z, by ^1H NMR. Purification by radial chromatography using a 2mm silica gel chromatotron plate, eluting with 50% CH_2Cl_2/50% petroleum ether gave Z-enollactone (**1.17Z**) as a white solid (194mg, 47%): mp 165-166 °C (petroleum ether); ^1H NMR ($CDCl_3$) δ 1.44 (t, J=7.1Hz, OCH_2CH_3), 4.43 (q, J=7.1Hz, OCH_2CH_3), 8.02 (s, **H-4**), 8.86 (s, **H-7**); ^{13}C NMR ($CDCl_3$) δ 14.1, 63.3, 99.0, 125.9, 127.2, 128.3, 129.1, 137.2, 140.7, 152.4, 162.4, 162.7; Anal. Calcd for $C_{12}H_7BrCl_2O_4$: C 39.4; H 1.9. Found: C 39.4; H 1.9. Further elution gave E-enollactone (**1.17E**) as a white solid (58mg, 14%): mp 154-155 °C (petroleum ether); IR (KBr) 1810, 1720 and 1620cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.41 (t, J=7.1Hz, OCH_2CH_3), 4.42 (q, J=7.1Hz, OCH_2CH_3), 8.08 (s, **H-4**), 8.77 (s, **H-7**); ^{13}C NMR ($CDCl_3$) δ 14.1, 63.3, 98.7, 125.5, 127.6, 127.9, 136.2, 137.5, 140.2, 147.7, 161.3, 162.4; HRMS (CI, M+1) Found 364.8980 (Calcd for $C_{12}H_8{}^{79}Br^{35}Cl_2O_4$ 364.8983).

METHOD B: $Ph_3P{=}CHCO_2Et$[E.03] (80mg, 0.23mmol, 1equiv) and 4,5-dichlorophthalic anhydride (**1.16**) (50mg, 0.23mmol, 1equiv) were stirred in CH_2Cl_2 (10mL) at 20 °C for 10min. Triethylamine (32μL, 0.23mmol, 1equiv), followed by Br_2 (8μL, 0.16mmol, 0.7equiv), were added and the solution was stirred for a further 30min at 20 °C. The solvent was evaporated and a ^1H NMR spectrum of the crude product revealed an isomer ratio of

20% E : 80% Z. Purification by radial chromatography using a 2mm silica gel chromatotron plate, eluting with 50% CH_2Cl_2/50% petroleum ether gave Z-enollactone (**1.17Z**) (21mg, 36%) and E-bromo enollactone (**1.17E**) (5mg, 8%): ^1H NMR ($CDCl_3$) as given above.

Crystal Data for of Ethyl Z-bromo-(4,5-dichloro-3-oxo-1,3-dihydroiso-benzofuran-1-ylidene)acetate (**1.17Z**).

$C_{12}H_7BrCl_2O_4$, colourless, crystallized from petroleum ether, crystal dimensions 0.14 x 0.03 x 0.04 mm, space group $P\bar{1}$, a = 6.272(2), b = 7.682(5), c = 14.661(6) Å, α = 92.98(4), β = 100.46(3), γ = 113.36(4)°, V = 631.7(6) Å^3, Z = 2, $F(000)$ = 360. Using 2.4° ω-scans at scan rate of 7.32° min^{-1}, 1650 unique reflections were collected in the range of $4 < 2\theta <$ 45° and 772 of these having $I > 3\sigma(I)$ were used in the structural analysis.

Data were recorded at 160 K on a Nicolet R3m four circle diffractometer using Mo - Kα radiation. Cell parameters were determined by least squares refinement of 24 accurately centred reflections. Crystal stability was monitored by recording check reflections and no significant variations were observed. Data were corrected for Lorentz-polarization effects and for absorption.

Direct methods revealed the position of the non-hydrogen atoms and the structure was refined by blocked-cascade least-squares techniques. Hydrogen atoms were inserted at calculated positions using a riding model with thermal parameters equal to 1.2 U of their carrier atoms. The refinement converged with R = 0.065 and R_W = 0.073 and a maximum least-squares shift/error of 0.001. The final difference fourier map showed no significant features. All programs used in the data collection and structure solution are contained in the SHELXTL (Version 4.1) package.

TABLE E.01: Atomic Coordinates ($x10^4$) and Isotropic Thermal Parameters ($Å^2x10^3$) for (**1.17Z**)

Atom	x	y	z	U^*_{eq}
Br	4630 (4)	8255 (3)	1147 (2)	16 (1)*
Cl (5)	-2135 (10)	12825 (8)	3898 (4)	31 (3)*
Cl (4)	-3060 (9)	15166 (7)	2276 (4)	25 (3)*
C (1)	1721 (33)	12314 (27)	530 (14)	14 (5)
C (2)	624 (33)	12597 (28)	1319 (13)	16 (5)
C (3)	-623 (31)	13724 (26)	1360 (13)	14 (5)
C (4)	-1472 (34)	13757 (28)	2167 (13)	17 (5)
C (5)	-1139 (35)	12720 (29)	2887 (14)	18 (5)
C (6)	59 (35)	11528 (29)	2786 (14)	20 (5)
C (7)	956 (32)	11508 (27)	1980 (13)	14 (5)
C (8)	2306 (35)	10448 (29)	1628 (15)	23 (5)
C (9)	3163 (31)	9192 (26)	1949 (13)	11 (4)
C (10)	3076 (35)	8533 (29)	2875 (14)	18 (5)
C (11)	4314 (36)	6615 (31)	3909 (14)	28 (6)
C (12)	6599 (38)	7786 (32)	4556 (15)	38 (6)
O (1)	1891 (23)	13051 (19)	-167 (9)	24 (7)*
O (2)	2644 (22)	11025 (17)	761 (9)	17 (6)*
O (3)	4205 (24)	7374 (21)	3015 (9)	26 (7)*
O (4)	2182 (26)	8997 (22)	3459 (10)	37 (8)*

*Equivalent isotropic U defined as one third of the trace of the orthogonalized U_{ij} tensor

TABLE E.02: Bond lengths (Å) for (**1.17Z**).

Br-C (9)	1.891 (22)	Cl (5)-C (5)	1.720 (23)
Cl (4)-C (4)	1.756 (26)	Cl (1)-C (2)	1.496 (32)
C (1)-O (1)	1.195 (25)	C (1)-O (2)	1.359 (28)
C (2)-O (3)	1.384 (34)	C (2)-C (7)	1.353 (30)
C (4)-O (5)	1.391 (30)	C (3)-C (4)	1.386 (30)
C (7)-C (8)	1.520 (35)	C (5)-C (6)	1.413 (37)
C (8)-C (9)	1.344 (34)	C (6)-C (7)	1.400 (31)
C (8)-O (2)	1.393 (26)	C (9)-C (10)	1.477 (28)
C (10)-O (3)	1.343 (31)	C (10)-O (4)	1.214 (30)
C (11)-O (12)	1.469 (25)	C (11)-O (3)	1.464 (26)

TABLE E.03: Bond angles (°) for (**1.17Z**).

C (2)-C (1)-O (1)	130.4 (23)	C (2)-C (1)-O (2)	106.2 (17)
O (1)-C (1)-O (2)	123.4 (21)	C (1)-C (2)-C (3)	126.0 (19)
C (1)-C (2)-C (7)	109.3 (21)	C (3)-C (2)-C (7)	124.7 (21)
Cl (4)-C (4)-C (5)	118.7 (17)	C (2)-C (3)-C (4)	115.3 (20)
Cl (5)-C (5)-C (4)	122.6 (20)	Cl (4)-C (4)-C (3)	118.2 (17)
C (4)-C (5)-C (6)	118.9 (20)	C (3)-C (4)-C (5)	123.1 (23)
C (5)-C (6)-C (7)	118.4 (20)	Cl (5)-C (5)-C (6)	118.5 (16)
C (2)-C (7)-C (6)	119.5 (23)	C (2)-C (7)-C (8)	106.8 (19)
C (6)-C (7)-C (8)	133.7 (20)	C (7)-C (8)-C (9)	135.9 (20)
C (7)-C (8)-O (2)	105.6 (18)	C (9)-C (8)-O (2)	118.5 (21)
Br-C (9)-C (8)	116.5 (16)	Br-C (9)-C (10)	118.5 (17)
C (8)-C (9)-C (10)	124.9 (21)	C (9)-C (10)-O (3)	110.9 (20)
C (9)-C (10)-O (4)	125.9 (23)	O (3)-C (10)-O (4)	123.1 (20)
C (1)-O (2)-C (8)	112.3 (17)	C (12)-C (11)-O (3)	110.1 (16)
		C (10)-O (3)-C (11)	117.9 (18)

TABLE E.04: Anisotropic Thermal Parameters ($Å^2x10^3$) for (**1.17Z**).

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
Br	15 (1)	21 (1)	17 (1)	2 (1)	6 (1)	11 (1)
Cl (5)	39 (4)	36 (4)	27 (4)	5 (3)	14 (3)	22 (3)
Cl (4)	23 (3)	29 (4)	30 (4)	1 (3)	10 (3)	16 (3)
O (1)	29 (9)	30 (9)	27 (9)	12 (7)	9 (7)	24 (8)
O (2)	24 (8)	4 (8)	23 (8)	1 (6)	7 (6)	5 (7)
O (3)	28 (9)	47 (10)	24 (9)	22 (8)	20 (7)	28 (8)
O (4)	42 (10)	49 (11)	27 (9)	6 (8)	13 (8)	24 (9)

The anisotropic temperature factor exponent takes the form:

$-2\pi^2 (h^2a^{*2}U_{11} + ... + 2hka^*b^*U_{12})$

TABLE E.05: Hydrogen Coordinates ($x10^4$) and Temperature Factors ($Å^2x10^3$) for (**1.17Z**).

Atom	x	y	z	U
H (3)	-898	14426	862	21
H (6)	248	10744	3256	25
H (11A)	3037	6641	4177	37
H (11B)	4152	5320	3806	37
H (12A)	6656	7286	5142	47
H (12B)	6763	9082	4658	47
H (12C)	7876	7758	4288	47

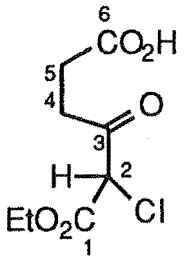
SECTION E.1.2

REACTION OF CHLORO ENOLLACTONES WITH WATER AND METHANOL:

General Method for the Formation of Acids (**1.42-1.44**):

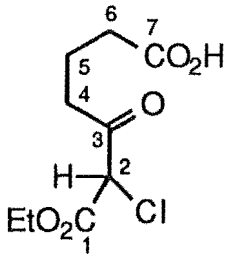
The succinic- and glutaric- chloro enollactones (**1.11-1.13**) reacted with atmospheric H_2O, over 3 weeks at 20 °C, to form acids (**1.42-1.44**), used subsequently without purification, which existed as oils.

1-Ethyl 2-chloro-3-hexandioate (**1.42**):



IR (film) 3000 and 1740cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.32 (t, J=7.1Hz, CH_3), 2.71 (t, J=6.5Hz, (**H-5**)$_2$), 3.05 (m, (**H-4**)$_2$), 4.30 (q, J=7.1Hz, OCH_2), 4.88 (s, **H-2**); ^{13}C NMR ($CDCl_3$) δ 13.82, 27.74, 33.43, 60.91, 63.29, 164.77, 177.80, 197.53; HRMS (M) Found 222.0287 (Calcd for $C_8H_{11}{}^{35}ClO_5$ 222.0295).

1-Ethyl 2-chloro-3-heptandioate (**1.43**):



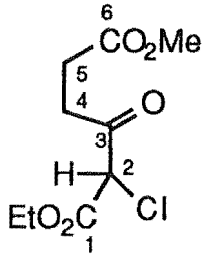
IR (film) 3000 and 1740cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.32 (t, J=7.1Hz, CH_3), 1.97 (quin, J=7.1Hz, (**H-5**)$_2$), 2.43 (t, J=7.2Hz, (**H-6**)$_2$), 2.83 (m, (**H-4**)$_2$), 4.30 (q, J=7.1Hz, OCH_2), 4.79 (s, **H-2**); ^{13}C NMR ($CDCl_3$) δ 13.93, 18.33, 32.37, 37.61, 60.78, 63.25, 164.95, 178.31, 198.38; HRMS (M) Found 238.0439 (Calcd for $C_9H_{13}{}^{37}ClO_5$ 238.0422), Found 236.0447 (Calcd for $C_9H_{13}{}^{35}ClO_5$ 236.0452).

The dimethyl glutaric chloro enollactone (**1.14**) also underwent this reaction.

General Method for the Formation of Methyl Esters (**1.45-1.47, 1.52**):

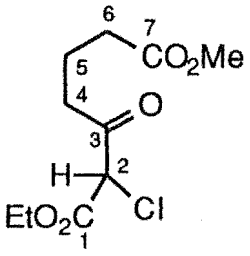
The succinic- and glutaric- chloro enollactones (**1.11-1.13**), the corresponding acids (**1.42-1.44**) and protio enollactone[E.05] (**1.51**) reacted, at 20 °C, with traces of methanol and acid (for example; on silica gel chromatotron plates) to form the corresponding methyl esters (**1.45-1.47, 1.52**, respectively). Purification by radial chromatography using silica gel chromatotron plates, eluting with the stated solvent system yielded the methyl esters (**1.45-1.47, 1.52**) as oils.

1-Ethyl 6-methyl 2-chloro-3-oxohexandioate (**1.45**):



Eluted with 85% petroleum ether/15% ethyl acetate; IR (film) 1740cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.33 (t, J=7.1Hz, CH_2CH_3), 2.67 (t, J=6.5Hz, (**H-5**)$_2$), 3.04 (m, (**H-4**)$_2$), 3.69 (s, OCH_3), 4.31 (q, J=7.1Hz, OCH_2), 4.87 (s, **H-2**); ^{13}C NMR ($CDCl_3$) δ 13.88, 27.79, 33.74, 51.92, 60.97, 63.20, 164.80, 172.38, 197.67; HRMS (M) Found 238.0384 (Calcd for $C_9H_{13}{}^{37}ClO_5$ 238.0422), Found 236.0450 (Calcd for $C_9H_{13}{}^{35}ClO_5$ 236.0452). Anal. Calcd for $C_9H_{13}ClO_5$: C 45.68; H 5.54. Found: C 45.68; H 5.63.

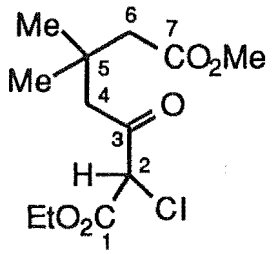
1-Ethyl 7-methyl 2-chloro-3-oxoheptandioate (**1.46**):



Eluted with 90% CH_2Cl_2/10% ethyl acetate; IR (film) 1730 and 1640cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.32 (t, J=7.1Hz, CH_2CH_3), 1.96 (quin, J=7.2Hz, (**H-5**)$_2$), 2.37 (t, J=7.2Hz, (**H-6**)$_2$), 2.81 (m, (**H-4**)$_2$), 3.68 (s, **H-2**), 4.29 (q, J=7.1Hz, OCH_2), 4.78 (s, OCH_3); ^{13}C NMR ($CDCl_3$) δ 13.89, 18.60, 32.51,

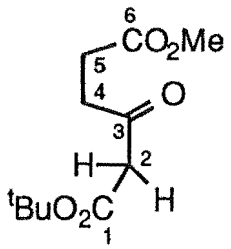
37.75, 51.61, 60.77, 63.17, 164.92, 173.26, 198.40; HRMS (M) Found 250.0616 (Calcd for $C_{10}H_{15}{}^{35}ClO_5$ 250.0630).

1-Ethyl 7-methyl 2-chloro-5,5-dimethyl-3-oxoheptandioate (**1.47**):



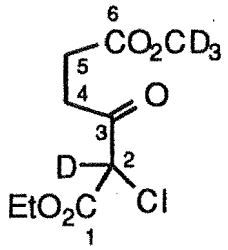
Eluted with 70% CH_2Cl_2/30% petroleum ether; FTIR (film) 1734cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.11 (s, C(C**H**$_3$)$_2$), 1.32 (t, J=7.2Hz, CH_2C**H**$_3$), 2.46 (AB$_q$, J_{AB}=14.9 Hz, 1H, (**H-6**)$_a$), 2.50 (AB$_q$, J_{AB}=14.9Hz, 1H, (**H-6**)$_b$), 2.84 (AB$_q$, J_{AB}=18.0Hz, 1H, (**H-4**)$_a$), 2.90 (AB$_q$, J_{AB}= 18.0Hz, 1H, (**H-4**)$_b$), 3.65 (s, OC**H**$_3$), 4.30 (q, J=7.2Hz, OC**H**$_2$), 4.81 (s, **H-2**): HRMS (M) Found 278.9903 (Calcd for $C_{12}H_{19}{}^{35}ClO_5$ 278.9903).

6-Methyl 1-(tert-butyl) 3-oxohexandioate (**1.52**):



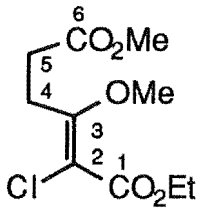
Eluted with 80% petroleum ether/20% ethyl acetate; bp 115-125 °C (1mm, colourless oil); IR (film) 1750 and 1730cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.48 (s, C(C**H**$_3$)$_3$), 2.62 (t, J=6.6Hz, (**H-5**)$_2$), 2.87 (t, J=6.6Hz, (**H-4**)$_2$), 3.40 (s, (**H-2**)$_2$), 3.68 (s, OC**H**$_3$); ^{13}C NMR ($CDCl_3$) δ 27.68, 27.97, 37.35, 50.60, 51.84, 82.10, 166.21, 172.87, 201.41; HRMS (M) Found 230.1122 (Calcd for $C_{11}H_{18}O_5$ 230.1154).

Reaction of chloro enollactone (**1.11**) with CD_3OD:



Chloro enollactone (**1.11**) (6mg, 0.029mmol) and a catalytic quantity of *p*-toluene sulphonic acid (PTSA) were dissolved in CD_3OD (0.7mL). After 24h, when all the enollactone had been converted to acid (**1.50**), the sample was filtered to remove PTSA and the solvent was evaporated: ^2H NMR ($CHCl_3$) δ 3.66 (OCD_3), 5.09 (CDCl).

Preparation of 1-Ethyl 6-methyl (E)-2-chloro-3-methoxyhex-2-enedioate (**1.53**):



Succinic-derived acid (**1.42**) (15mg, 0.07mmol, 1equiv) was dissolved in ether (2mL) and an excess of freshly distilled CH_2N_2 in ether was added. The excess CH_2N_2 was allowed to evaporate and the residue was purified by radial chromatography using a 1mm silica gel chromatotron plate, eluting with 95% CH_2Cl_2/5% ethyl acetate to yield an oil which is tentatively assigned as the alkene (**1.53**) (6mg, 36%): IR (film) 1740, 1670 and 1590cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.33 (t, J=7.1Hz, CH_2CH_3), 2.59 (m, (**H-5**)$_2$), 3.19 (m, (**H-4**)$_2$), 3.71 (s, OCH_3), 3.87 (s, OCH_3), 4.25 (q, J=7.1Hz, OCH_2); ^{13}C NMR ($CDCl_3$) δ 14.16, 24.10, 31.61, 51.95, 56.24, 61.56, 104.93, 164.01, 166.21, 172.32; HRMS (M) Found 252.0574 (Calcd for $C_{10}H_{15}{}^{37}ClO_5$ 252.0600), Found 250.0602 (Calcd for $C_{10}H_{15}{}^{35}ClO_5$ 250.0630).

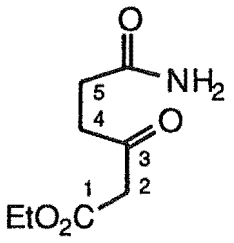
SECTION E.2

CHAPTER 2 EXPERIMENTAL

SECTION E.2.1

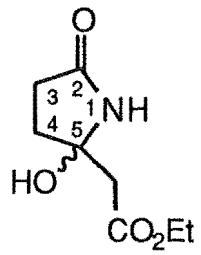
PREPARATION OF KETO-AMIDES AND HYDROXY LACTAMS (**2.37-2.50**)

*Preparation of Ethyl 5-carbamoyl-3-oxopentanoate (**2.37**):*



Ammonia (0.5mL of 22.5mg/mL solution in ethanol, 0.66mmol, 8equiv) was added to enollactone[E.05] (**2.33a**) (15mg, 0.08mmol, 1equiv) in CH_2Cl_2 (3mL) and the solution was stirred at 20 °C for 5h. The solvent was evaporated to yield keto-amide (**2.37**) as an oil, which was used in subsequent steps without further purification: Yield 17mg, quant; ^1H NMR ($CDCl_3$) δ1.28 (t, J=7.1Hz, C**H**$_3$), 2.53 (t, J=6.5Hz, (**H-4**)$_2$), 2.92 (t, J=6.5Hz, (**H-5**)$_2$), 3.50 (s, (**H-2**)$_2$), 4.20 (q, J=7.2Hz, OC**H**$_2$), 5.50 (bs, N**H**$_2$); ^{13}C NMR ($CDCl_3$) δ 14.13, 29.05, 37.79, 49.17, 61.42, 167.06, 173.84, 201.88; HRMS (M) Found 187.0842 (Calcd for $C_8H_{13}NO_4$ 187.0845).

*Preparation of (5R,S) 5-(Ethoxycarbonylmethyl)-5-hydroxy-2-pyrrolidinone (**2.48**):*



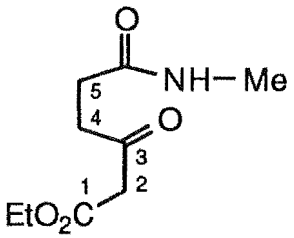
Ammonia (0.7mL of 22.5mg/mL solution in ethanol, 0.91mmol, 11equiv) was added to enollactone[E.05] (**2.33a**) (15mg, 0.88mmol, 1equiv) and the solution was stirred at 20 °C for 5h. The solvent was evaporated to yield the hydroxy lactam (**2.48**) as an oil, which was used in subsequent steps without further purification: Yield 17mg, quant; IR (film) 3400 and 1700cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.30 (t, J=7.2Hz, C**H**$_3$), 2.11 (m, 1H), 2.28 (m, 1H), 2.34 (m, 1H), 2.60

(m, 1H), 2.75 (AB_q, J_{AB}=17.0Hz, 1H, CH_aCO_2Et), 2.93 (AB_q, J_{AB}=17.0Hz, 1H, CH_bCO_2Et), 4.23 (q, J=7.2Hz, OCH_2), 6.60 (bs, NH); ^{13}C NMR ($CDCl_3$) δ 13.93, 29.19, 34.60, 45.09, 60.95, 86.36, 170.55, 177.82; HRMS (M) Found 187.0848 (Calcd for $C_8H_{13}NO_4$ 187.0845).

General Method for the Preparation of Amine-Derived Keto-Amides (**2.38-2.41**) and Hydroxy Lactam (**2.49**):

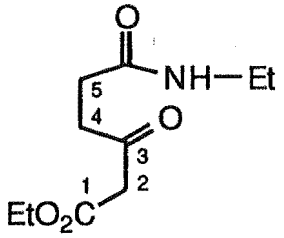
The indicated amine (stated amount) was added to the appropriate enollactone[E.05-E.06] (**2.33a-b, 2.33d**) (1equiv) dissolved in CH_2Cl_2 or 1, 2-dichloroethane and the solution was stirred for 16h at 20 °C. The solvent was evaporated at 20mm and finally at 1mm to quantitatively yield the keto-amide (**2.38-2.41**) or hydroxy lactam (**2.49**), which was used in subsequent steps without further purification.

Ethyl 5-(N-methylcarbamoyl)-3-oxopentanoate (**2.38**):



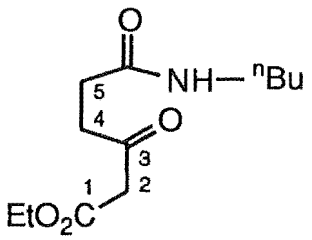
General method with enollactone (**2.33a**) (20mg, 0.12mmol) and methylamine (53μL of 3.91M solution in 1, 2-dichloroethane, 0.22mmol, 1.8equiv), in 1, 2-dichloroethane (5mL): Yield 25mg, white solid, quant; IR (KBr) 3350, 1750, 1720, 1650 and 1570cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.28 (t, J=7.1Hz, CH_2CH_3), 2.47 (t, J=6.5Hz, (**H-4**)$_2$), 2.79 (d, J=4.8Hz, NCH_3), 2.92 (t, J=6.5Hz, (**H-5**)$_2$), 3.50 (s, (**H-2**)$_2$), 4.20 (q, J=7.1Hz, OCH_2), 5.69 (bs, NH); ^{13}C NMR ($CDCl_3$) δ 14.04, 26.33, 29.63, 38.05, 49.17, 61.40, 167.10, 172.01, 202.17; HRMS (M) Found 201.1006 (Calcd for $C_9H_{15}NO_4$ 201.1002).

Ethyl 5-(N-ethylcarbamoyl)-3-oxopentanoate (**2.39**):



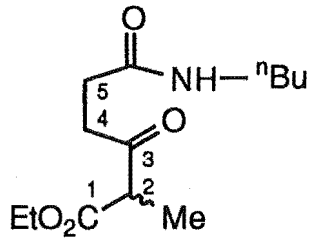
General method with enollactone (**2.33a**) (10mg, 0.06mmol) and ethylamine (57μL of 1.53M solution in CH_2Cl_2, 0.11mmol, 1.8equiv), in CH_2Cl_2 (5mL): Yield 13mg, white solid, quant; IR (KBr) 3425, 3325, 1755, 1730, 1660 and 1560cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.13 (t, J=7.2Hz, NCH_2CH_3), 1.28 (t, J=7.2Hz, OCH_2CH_3), 2.45 (t, J=6.5Hz, (**H-4**)$_2$), 2.91 (t, J=6.5Hz, (**H-5**)$_2$), 3.27 (m, NCH_2), 3.50 (s, (**H-2**)$_2$), 4.20 (q, J=7.2Hz, OCH_2), 5.27 (bs, **NH**); ^{13}C NMR ($CDCl_3$) δ 13.94, 14.58, 29.65, 34.32, 37.96, 49.09, 61.26, 167.05, 171.17, 202.10; HRMS (M) Found 215.1158 (Calcd for $C_{10}H_{17}NO_4$ 215.1158).

Ethyl 5-(N-(1-butyl)-carbamoyl)-3-oxopentanoate (**2.40**):



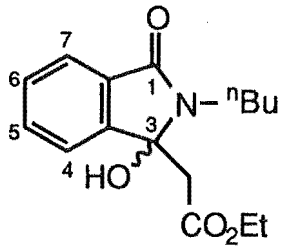
General method with enollactone (**2.33a**) (50mg, 0.29mmol) and butylamine (30μL, 0.29mmol, 1equiv), in CH_2Cl_2 (5mL): Yield 83mg, white solid, quant; IR (KBr) 3300, 1775, 1720, 1650 and 1560cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.92 (t, J=7.2Hz, $CH_2CH_2CH_3$), 1.28 (t, J=7.1Hz, OCH_2CH_3), 1.35 (m, $NCH_2CH_2CH_2$), 1.47 (m, NCH_2CH_2), 2.46 (t, J=6.5Hz, (**H-4**)$_2$), 2.91 (t, J=6.5Hz, (**H-5**)$_2$), 3.22 (m, NCH_2), 3.50 (s, (**H-2**)$_2$), 4.20 (q, J=7.1Hz, OCH_2), 5.57 (bs, **NH**); ^{13}C NMR ($CDCl_3$) δ 13.64, 14.01, 19.95, 29.80, 31.54, 38.05, 39.30, 49.16, 61.33, 167.07, 171.24, 202.08; HRMS (M) Found 243.1470 (Calcd for $C_{12}H_{21}NO_4$ 243.1471).

Ethyl (2R,S) 2-methyl 5-(N-(1-butyl)-carbamoyl)-3-oxopentanoate (**2.41**):



General method with methyl enollactone (**2.33b**) (30mg, 0.16mmol) and butylamine (17μL, 0.16mmol, 1equiv), in CH_2Cl_2 (5mL): Yield 43mg, oil, quant; IR (film) 3325, 1750, 1725, 1660 and 1565cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.91 (t, J=7.2Hz, $CH_2CH_2CH_3$), 1.27 (t, J=7.1Hz, OCH_2CH_3), 1.32 (m, $NCH_2CH_2CH_2$), 1.35 (d, J=7.2Hz, $CHCH_3$), 1.47 (m, NCH_2CH_2), 2.45 (m, **(H-4)**$_2$), 2.91 (t, J=6.5Hz, **(H-5)**$_2$), 3.22 (m, NCH_2), 3.58 (q, J=7.2Hz, $CHCH_3$), 4.19 (q, J=7.1Hz, OCH_2), 5.75 (bs, **NH**); ^{13}C NMR ($CDCl_3$) δ 12.72, 13.67, 14.03, 19.98, 29.96, 31.59, 36.71, 39.32, 52.74, 61.37, 170.47, 171.38, 205.28; HRMS (M) Found 257.1620 (Calcd for $C_{13}H_{23}NO_4$ 257.1628).

2-Butyl (3R,S) 3-ethoxycarbonylmethyl-3-hydroxyisoindolone (**2.49**):

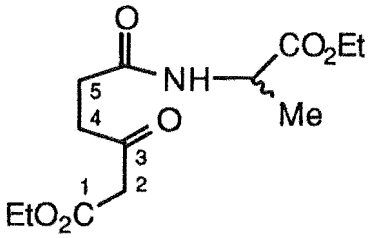


General method with phthalic enollactone (**2.33d**) (100mg, 0.52mmol) and butylamine (68μL, 0.67mmol, 1.3equiv), in CH_2Cl_2 (15mL): Yield 170mg, white solid, quant; IR (KBr) 3350, 1750, 1680 and 1630cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.93 (t, J=7.3Hz, $CH_2CH_2CH_3$), 1.08 (t, J=7.2Hz, OCH_2CH_3), 1.36 (sextet, J=7.4Hz, $NCH_2CH_2CH_2$), 1.62 (m, NCH_2CH_2), 2.99 (AB_q, J_{AB}=15.7Hz, 1H, COH-CH_a), 3.13 (AB_q, J_{AB}=15.7Hz, 1H, COH-CH_b), 3.20 (m, 1H, NCH_a), 3.51 (m, 1H, NCH_b), 4.09 (q, J=7.2Hz, OCH_2), 7.44 (m, **(Ph)**$_1$), 7.53 (m **(Ph)**$_2$), 7.65 (dt, J=1.0, 7.4Hz, **(Ph)**$_1$); ^{13}C NMR ($CDCl_3$) δ 13.76, 13.84, 20.57, 31.17, 38.96, 41.50, 61.13, 88.33, 121.72, 123.15, 129.66, 131.13 132.11, 146.32, 167.28, 169.78; HRMS (M) Found 291.1473 (Calcd for $C_{16}H_{21}NO_4$ 291.1471).

General Method for the Preparation of Amino Acid-Derived Keto-Amides (**2.42-2.47**) and Hydroxy Lactam (**2.50**):

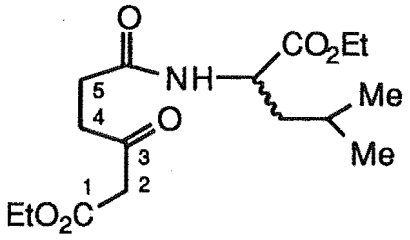
The indicated amino-acid ethylester hydrochloride (stated amount) and triethylamine (stated amount) were added to the appropriate enollactone[E.05-E.06] (**2.33a-d**) (1equiv) dissolved in CH_2Cl_2 and the mixture was stirred for 16h at 20 °C, during which time homogeneity was achieved. The solution was transferred to a separating funnel and washed with water (10mL). The organic layer was dried ($MgSO_4$) and the solvent evaporated at 20mm and finally at 1mm to yield the keto-amide (**2.42-2.47**) or hydroxy lactam (**2.50**), which was used in subsequent steps without further purification.

Ethyl (6'R,S) 5-(N-(1-ethoxycarbonylethyl)carbamoyl)-3-oxopentanoate (**2.42**):



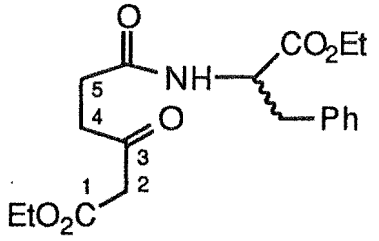
General method with enollactone (**2.33a**) (80mg, 0.47mmol), (R,S)-alanine ethylester hydrochloride[E.07] (94mg, 0.61mmol, 1.3equiv) and triethylamine (81µL, 0.61mmol, 1.3equiv), in CH_2Cl_2 (8mL): Yield 99mg, white solid, 73%; IR (KBr) 3325, 1760, 1720, 1650 and 1560cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.28 (t, J=7.1Hz, OCH_2CH_3), 1.28 (t, J=7.2Hz, OCH_2CH_3), 1.39 (d, J=7.2Hz $CHCH_3$), 2.53 (t, J=6.5Hz, (**H-4**)$_2$), 2.91 (m, (**H-5**)$_2$), 3.50 (s, (**H-2**)$_2$), 4.20 (q, J=7.2Hz, OCH_2), 4.20 (q, J=7.1Hz, OCH_2), 4.53 (m, $CHCH_3$), 6.15 (bd, J=6.8Hz, NH); ^{13}C NMR ($CDCl_3$) δ 14.09, 14.11, 18.46, 29.65, 37.78, 48.23, 49.26, 61.42, 61.50, 167.07, 170.86, 173.01, 201.77; HRMS (M) Found 287.1376 (Calcd for $C_{13}H_{21}NO_6$ 287.1369).

Ethyl (6'R,S) 5-(N-(1-ethoxycarbonyl-3-methylbutyl)carbamoyl)-3-oxopentanoate (**2.43**):



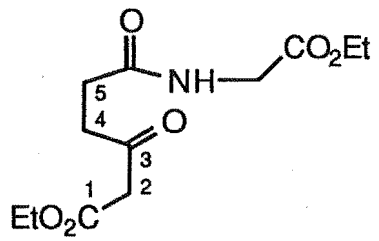
General method with enollactone (**2.33a**) (80mg, 0.47mmol), (R,S)-leucine ethylester hydrochloride[E.07] (120mg, 0.61mmol, 1.3equiv) and triethylamine (81μL, 0.61mmol, 1.3equiv), in CH_2Cl_2 (8mL): Yield 164mg, white solid, quant; IR (film) 3350, 1750, 1660 and 1550cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.93 (d, J=1.5Hz, CHC**H**$_3$), 0.95 (d, J=1.5Hz, CHC**H**$_3$), 1.28 (t, J=7.1Hz, OCH$_2$C**H**$_3$), 1.28 (t, J=7.1Hz, OCH$_2$C**H**$_3$), 1.60 (m, 3H, NCHC**H**$_2$CH), 2.53 (t, J=6.3Hz (**H-4**)$_2$), 2.91 (m, (**H-5**)$_2$), 3.49 (s, (**H-2**)$_2$), 4.18 (q, J=7.1Hz, OC**H**$_2$), 4.19 (q, J=7.1Hz, OC**H**$_2$), 4.58 (m, NC**H**), 5.96 (bd, J=7.6Hz, N**H**); ^{13}C NMR ($CDCl_3$) δ 14.05, 14.09, 21.97, 22.72, 24.80, 29.61, 37.81, 41.65, 49.19, 50.86, 61.27, 61.37, 167.05, 171.12, 172.99, 201.70; HRMS (M) Found 329.1837 (Calcd for $C_{16}H_{27}NO_6$ 329.1839).

Ethyl (6'R,S) 5-(N-(1-ethoxycarbonyl-2-phenylethyl)carbamoyl)-3-oxopentanoate (**2.44**):



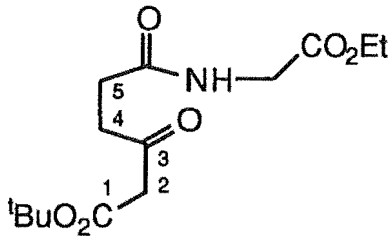
General method with enollactone (**2.33a**) (80mg, 0.47mmol), (R,S)-phenylalanine ethylester hydrochloride[E.07] (140mg, 0.61mmol, 1.3equiv) and triethylamine (81μL, 0.61mmol, 1.3equiv), in CH_2Cl_2 (8mL): Yield 165mg, white solid, 97%; IR (KBr) 3340, 1740, 1720, 1655 and 1530cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.24 (t, J=7.1Hz, C**H**$_3$), 1.28 (t, J=7.1Hz, C**H**$_3$), 2.49 (dt, J=1.7, 6.6Hz, (**H-4**)$_2$), 2.88 (m, (**H-5**)$_2$), 3.11 (dd, J=2.2, 5.8Hz, C**H**$_2$Ph), 3.48 (s, (**H-2**)$_2$), 4.17 (q, J=7.1Hz, OC**H**$_2$), 4.20 (q, J=7.1Hz, OC**H**$_2$), 4.82 (m, NC**H**), 6.03 (bd, J=7.2Hz, N**H**), 7.12 (m, (**Ph**)$_2$), 7.27 (m, (**Ph**)$_3$); ^{13}C NMR ($CDCl_3$) δ 14.05, 14.05, 29.55, 37.66, 37.89, 49.19. 53.21, 61.36, 61.44, 127.02, 128.46, 129.33, 135.84, 167.03, 170.84, 171.41, 201.58; HRMS (M) Found 363.1681 (Calcd for $C_{19}H_{25}NO_6$ 363.1683).

Ethyl 5-(N-(1-ethoxycarbonylmethyl)carbamoyl)-3-oxopentanoate (**2.45**):



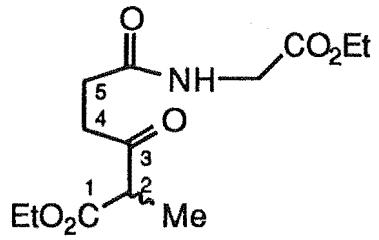
General method with enollactone (**2.33a**) (47mg, 0.28mmol), glycine ethylester hydrochloride (50mg, 0.36mmol, 1.3equiv) and triethylamine (47μL, 0.36mmol, 1.3equiv), in CH_2Cl_2 (5mL): Yield 66mg, white solid, 88%; mp 66.5-68.5 °C (ethyl acetate/petroleum ether, white crystals); IR (KBr) 3325, 1760, 1720, 1660 and 1560cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.26 (t, J=7.1Hz, C**H$_3$**), 1.29 (t, J=7.1Hz, C**H$_3$**), 2.56 (t, J=6.5Hz (**H-4**)$_2$), 2.92 (t, J=6.5Hz, (**H-5**)$_2$), 3.50 (s, (**H-2**)$_2$), 4.01 (d, J=5.2Hz, NC**H$_2$**), 4.20 (q, J=7.1Hz, OC**H$_2$**), 4.22 (q, J=7.1Hz, OC**H$_2$**), 6.11 (bs, N**H**); ^{13}C NMR ($CDCl_3$) δ 13.94, 13.99, 29.27, 37.69, 41.32, 49.07, 61.26, 61.30, 166.99, 169.80, 171.60, 201.80; HRMS (M) Found 273.1214 (Calcd for $C_{12}H_{19}NO_6$ 273.1213). Anal. Calcd for $C_{12}H_{19}NO_6$: C 52.74; H 7.01; N 5.13. Found: C 52.59; H 7.01; N 5.01.

Tert-*butyl 5-(N-(1-ethoxycarbonylmethyl)carbamoyl)-3-oxopentanoate* (**2.46**):



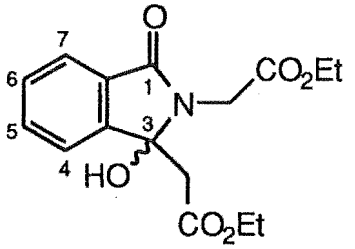
General method with *tert*-butyl enollactone (**2.33c**) (150mg, 0.76mmol), glycine ethylester hydrochloride (106mg, 0.76mmol, 1equiv) and triethylamine (98μL, 0.76mmol, 1equiv), in CH_2Cl_2 (5mL): Yield 199mg, yellow oil, 87%; IR (film) 3350, 1735, 1715, 1660 and 1540cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.29 (t, J=7.2Hz, OCH$_2$C**H$_3$**), 1.47 (s, C(C**H$_3$**)$_3$), 2.55 (t, J=6.5Hz, (**H-4**)$_2$), 2.91 (t, J=6.5Hz, (**H-5**)$_2$), 3.41 (s, (**H-2**)$_2$), 4.01 (d, J=5.2Hz, NC**H$_2$**), 4.22 (q, J=7.2Hz, OC**H$_2$**), 6.13 (bs, N**H**); ^{13}C NMR ($CDCl_3$) δ 13.83, 27.65, 29.07, 37.52, 41.14, 50.16, 61.04, 81.64, 166.17, 169.71, 171.77, 202.17; HRMS (M-18) Found 283.1422 (Calcd for $C_{14}H_{21}NO_5$ 283.1420).

Ethyl (2R,S) 2-methyl 5-(N-(1-ethoxycarbonylmethyl)carbamoyl)-3-oxopentanoate **(2.47)**:



General method with methyl enollactone (**2.33b**) (85mg, 0.46mmol), glycine ethylester hydrochloride (79mg, 0.57mmol, 1.2equiv) and triethylamine (75μL, 0.57mmol, 1.2equiv), in CH_2Cl_2 (7mL): Yield 116mg, colourless oil, 88%; IR (film) 3375, 1750, 1730, 1660 and 1550cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.27 (t, J=7.1Hz, OCH_2CH_3), 1.29 (t, J=7.2Hz, OCH_2CH_3), 1.36 (d, J=7.1Hz, $CHCH_3$), 2.55 (m, **(H-4)**$_2$), 2.93 (t, J=6.4Hz, **(H-5)**$_2$), 3.58 (q, J=7.2Hz, $CHCH_3$), 4.01 (d, J=5.2Hz, NCH_2), 4.19 (q, J=7.1Hz, OCH_2), 4.22 (q, J=7.2Hz, OCH_2), 6.11 (bs, NH); ^{13}C NMR ($CDCl_3$) δ 12.55, 13.87, 13.92, 29.29, 36.23, 41.24, 52.56, 61.21, 169.78, 170.31, 171.70, 204.86; HRMS (M) Found 287.1366 (Calcd for $C_{13}H_{21}NO_6$ 287.1369).

(3R,S) 2-ethoxycarbonylmethyl-3-ethoxycarbonylmethyl-3-hydroxyisoindolone **(2.50)**:



General method with phthalic enollactone (**2.33d**) (100mg, 0.52mmol), glycine ethylester hydrochloride (109mg, 0.78mmol, 1.5equiv) and triethylamine (102μL, 0.78mmol, 1.5equiv), in CH_2Cl_2 (5mL): Yield 131mg, colourless oil, 79%; IR (film) 3400, 1750, 1720 and 1630cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.06 (t, J=7.1Hz, CH_3), 1.31 (t, J=7.2Hz, CH_3), 3.07 (AB_q, J_{AB}=15.3Hz, 1H, COH-CH_a), 3.14 (AB_q, J_{AB}=15.3Hz , 1H, COH-CH_b), 4.00 (q, J=7.2Hz, OCH_2), 4.16 (AB_q, J_{AB}=17.9Hz, 1H, NCH_a), 4.23 (q, J=7.1Hz, OCH_2), 4.53 (AB_q, J_{AB}=17.9Hz, 1H, NCH_b), 7.52 (m, **(Ph)**$_1$), 7.62 (m, **(Ph)**$_2$), 7.81 (dt, J=1.0, 7.4Hz, **(Ph)**$_1$); ^{13}C NMR ($CDCl_3$) δ 13.71, 13.98, 40.35, 41.78, 60.86, 61.66, 87.92, 122.13, 123.37, 129.71, 130.14, 132.59, 146.55, 167.22, 168.84, 170.19; HRMS (M) Found 321.1208 (Calcd for $C_{16}H_{19}NO_6$ 321.1213).

SECTION E.2.2

PREPARATION OF ENAMINO ESTERS (**2.63-2.75**)

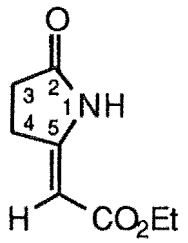
General Methods for the Preparation of Enamino Esters (**2.63-2.75**):

GENERAL METHOD A: The appropriate keto-amide or hydroxy lactam (**2.37-2.50**) (stated amount, 1equiv) and a catalytic quantity of PTSA, dissolved in 1, 2-dichloroethane, were refluxed with azeotropic removal of H_2O for the indicated time. The solution was cooled to 20 °C, washed with H_2O (15mL), dried ($MgSO_4$) and the solvent evaporated to yield the enamino ester (**2.63-2.75**).

GENERAL METHOD B: As for General Method A except that the solvent used was benzene.

GENERAL METHOD C: The appropriate enollactone[E.05] (**2.33a**) or keto-amide (**2.37**) (stated amount, 1equiv) and the indicated amine (stated amount) were dissolved in 1, 2-dichloroethane. Activated 4Å molecular sieves were added and the solution was stirred at 65 °C for 3 days, then was filtered and the solvent was evaporated to yield the enamino ester (**2.63-2.66**).

(Z)-5-Ethoxycarbonylmethylidene-2-pyrrolidinone[E.08] (**2.63Z**):

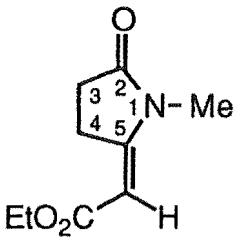


General method C with hydroxy lactam (**2.48**) (12mg, 0.064mmol) in 1, 2-dichloroethane (5mL): Yield 9mg, white solid, 83%; mp 79-81 °C (ethanol/H_2O) (Lit[E.08] 79-80 °C); IR (KBr) 3280, 1760, 1735, 1685, 1630 and 1610cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.28 (t, J=7.1Hz, CH_3), 2.52 (m, (**H-3**)$_2$), 2.87 (m, (**H-4**)$_2$), 4.17 (q, J=7.1Hz, OCH_2), 5.00 (t, J=1.5Hz, =C**H**), 9.88 (bs, N**H**); ^{13}C NMR ($CDCl_3$) δ 14.35, 26.04, 27.71, 59.90, 90.20, 157.41, 168.11, 177.36; HRMS (M) Found 169.0739

(Calcd for $C_8H_{11}NO_3$ 169.0739). ^1H NMR ($CDCl_3$) also showed the presence of less than 5% of the E isomer: δ 5.30 (t, J=2.0Hz, =C**H**).

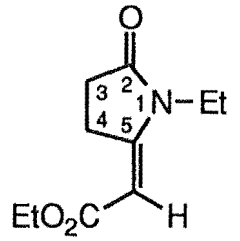
General method B with keto-amide (**2.37**) (17mg, 0.091mmol) and a catalytic quantity of PTSA, in benzene (15mL), and a reflux time of 2h gave comparable results: Yield 2mg, oil, 13%; ^1H NMR ($CDCl_3$) as given above.

1-Methyl (E)-5-ethoxycarbonylmethylidene-2-pyrrolidinone[E.09] *(**2.64**):*



General method C with enollactone[E.05] (**2.33a**) (20mg, 0.12mmol) and methylamine (52μL of 3.91M solution in 1, 2-dichloroethane, 0.20mmol, 1.7equiv), in 1, 2-dichloroethane (5mL), and with heating for 4 days: Yield 20mg, white solid, 93%; ^1H NMR ($CDCl_3$) δ 1.29 (t, J=7.1Hz, CH_2CH_3), 2.57 (m, (**H-3**)$_2$), 3.00 (s, NCH_3), 3.24 (m, (**H-4**)$_2$), 4.17 (q, J=7.1Hz, OCH_2), 5.19 (t, J=1.9Hz, =C**H**).

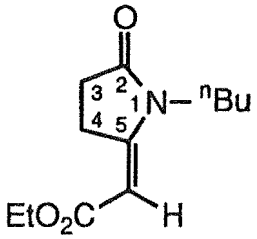
*1-Ethyl (E)-5-ethoxycarbonylmethylidene-2-pyrrolidinone (**2.65**):*



General method B with keto-amide (**2.39**) (67mg, 0.29mmol) and a catalytic quantity of PTSA, in benzene (15mL), and a reflux time of 3h: Yield 41mg, white solid, 71%; mp 158-159 °C; IR (KBr) 1745, 1715 and 1630cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.16 (t, J=7.2Hz, NCH_2CH_3), 1.30 (t, J=7.1Hz, OCH_2CH_3), 2.55 (m, (**H-3**)$_2$), 3.23 (m, (**H-4**)$_2$), 3.58 (q, J=7.2Hz, NCH_2), 4.17 (q, J=7.1Hz, OCH_2), 5.23 (t, J=1.9Hz, =C**H**); ^{13}C NMR ($CDCl_3$) δ 11.63, 14.39, 24.68, 27.98, 35.23, 59.48, 91.21, 159.50, 167.33, 176.61; HRMS (M) Found 197.1054 (Calcd for $C_{10}H_{15}NO_3$ 197.1053). Anal. Calcd for $C_{10}H_{15}NO_3$: C 60.90; H 7.67; N 7.10. Found: C 60.65; H 7.77; N 6.63.

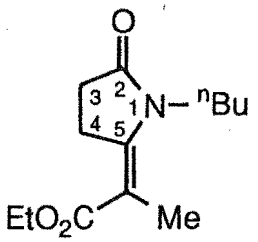
General method C with enollactone (**2.33a**) (50mg, 0.29mmol) and ethylamine (0.25μL, 0.38mmol, 1.3equiv), in 1, 2-dichloroethane (10mL) gave comparable results: Yield 41mg, white solid, 71%; ^{1}H NMR ($CDCl_3$) as given above. ^{1}H NMR ($CDCl_3$) also showed the presence of less than 5% of the Z isomer: δ 5.02 (t, J=1.5Hz, =C**H**).

*1-Butyl (E)-5-ethoxycarbonylmethylidene-2-pyrrolidinone (**2.66**):*

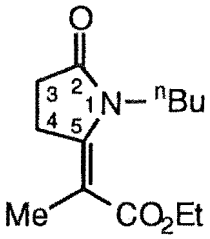


General method C with enollactone (**2.33a**) (50mg, 0.29mmol) and butylamine (39μL, 0.38mmol, 1.3equiv) in 1, 2-dichloroethane (10mL): Yield 65mg, oil, 100%; bp 175 °C (1mm); IR (film) 1750, 1715 and 1630cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.94 (t, J=7.3Hz, $CH_2CH_2CH_3$), 1.30 (t, J=7.1Hz, OCH_2CH_3), 1.32 (m, $NCH_2CH_2CH_2$), 1.54 (m, NCH_2CH_2), 2.55 (m, (**H-3**)$_2$), 3.23 (m, (**H-4**)$_2$), 3.51 (t, J=7.6Hz, NCH_2), 4.17 (q, J=7.1Hz, OCH_2), 5.21 (t, J=1.9Hz, =C**H**); ^{13}C NMR ($CDCl_3$) δ 13.56, 14.33, 20.06, 24.62, 27.84, 28.30, 40.23, 59.40, 91.22, 159.83, 167.26, 176.79; HRMS (M) Found 225.1368 (Calcd for $C_{12}H_{19}NO_3$ 225.1366). Anal. Calcd for $C_{12}H_{19}NO_3$: C 63.98; H 8.50; N 6.22. Found: C 63.73; H 8.36; N 6.37. ^{1}H NMR ($CDCl_3$) also showed the presence of less than 5% of the Z isomer: δ 5.02 (t, J=1.5Hz, =C**H**), which was converted to the E isomer upon heating for a further 3 days.

*1-Butyl (E)- and (Z)-5-(1-ethoxycarbonylethylidene)-2-pyrrolidinone (**2.67E** and **2.67Z**):*



2.67E



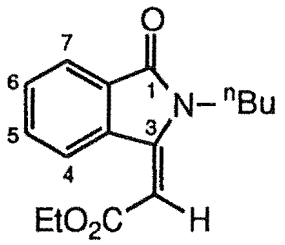
2.67Z

General method A with keto-amide (**2.41**) (112mg, 0.44mmol) and a catalytic quantity of PTSA, in 1, 2-dichloroethane (15mL), and a reflux time of 10h gave a mixture containing E- and Z-enamino esters (**2.67E** and **2.67Z**, respectively) in the ratio of 84% E : 16% Z, by ^{1}H

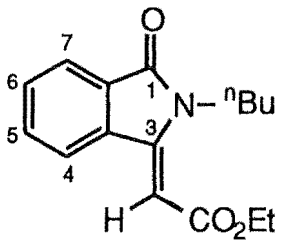
NMR. Purification by radial chromatography using a 1mm silica gel chromatotron plate, eluting with 75% petroleum ether/25% ethyl acetate gave E-enamino ester (**2.67E**) as an oil: 51mg, 50%; unstable to distillation; IR (film) 1740, 1710 and 1620cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.94 (t, J=7.3Hz, $CH_2CH_2CH_3$), 1.31 (t, J=7.1Hz, OCH_2CH_3), 1.31 (m, $NCH_2CH_2CH_2$), 1.55 (m, NCH_2CH_2), 2.06 (t, J=1.2Hz, $=CCH_3$), 2.47 (m, **(H-3)**$_2$), 3.13 (m, **(H-4)**$_2$), 3.78 (t, J=7.7Hz, NCH_2), 4.19 (q, J=7.1Hz, OCH_2); ^{13}C NMR ($CDCl_3$) δ 13.48, 13.70, 14.35, 19.84, 27.96, 28.68, 30.76, 42.63, 60.17, 101.44, 153.11, 169.25, 178.56; HRMS (M) Found 239.1521 (Calcd for $C_{13}H_{21}NO_3$ 239.1522). Further elution gave Z-enamino ester (**2.67Z**) as an oil: 10mg, 9%; IR (film) 1740, 1710 and 1630cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J=7.2Hz, $CH_2CH_2CH_3$), 1.23 (m, $NCH_2CH_2CH_2$), 1.32 (t, J=7.2Hz, OCH_2CH_3), 1.35 (m, NCH_2CH_2), 1.91 (t, J=1.1Hz, $=CCH_3$), 2.51 (m, **(H-3)**$_2$), 2.64 (m, **(H-4)**$_2$), 3.73 (t, J=7.5Hz, NCH_2), 4.21 (q, J=7.2Hz, OCH_2); ^{13}C NMR ($CDCl_3$) δ 13.76, 14.26, 16.46, 19.94, 25.36, 28.13, 28.49, 41.47, 60.68, 101.23, 143.84, 168.73, 177.40; HRMS (M) Found 239.1524 (Calcd for $C_{13}H_{21}NO_3$ 239.1522).

General method B with keto-amide (**2.41**) (42mg, 0.16mmol) and a catalytic quantity of PTSA, in benzene (10mL), and a reflux time of 4h gave, after radial chromatography: 9mg, oil, 23% (82% E (**2.67E**) : 18% Z (**2.67Z**), by ^{1}H NMR); ^{1}H NMR ($CDCl_3$) as given above.

*2-Butyl (E)- and (Z)-3-ethoxycarbonylmethylidene-isoindolone (**2.74E** and **2.74E**):*



2.74E



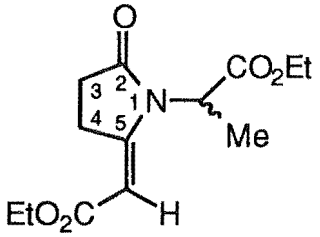
2.74Z

General method A with hydroxy lactam (**2.49**) (150mg, 0.51mmol) and a catalytic quantity of PTSA, in 1, 2-dichloroethane (15mL), and a reflux time of 3h gave a mixture of E- and Z-enamino esters (**2.74E** and **2.74Z**, respectively) (86% E : 24% Z, by ^{1}H NMR): Yield 134mg, oil, 76%. The E isomer (**2.74E**) was isolated by crystallization (ethanol/H_2O): mp 72-73 °C; IR (KBr) 1725 and 1635cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.97 (t, J=7.3Hz, $CH_2CH_2CH_3$), 1.37 (t, J=7.1Hz, OCH_2CH_3), 1.39 (m, $NCH_2CH_2CH_2$), 1.65 (m, NCH_2CH_2), 3.79 (t, J=7.4Hz, NCH_2), 4.29 (q,

J=7.1Hz, OCH_2), 5.71 (s, =CH), 7.57 (dt, J=1.1, 7.4Hz, **H-4**), 7.65 (dt, J=1.4, 7.6Hz, **H-5**), 7.85 (dd, J=0.9, 6.3Hz, **H-3**), 9.06 (d, J=7.8Hz, **H-4**); ^{13}C NMR ($CDCl_3$) δ 13.60, 14.23, 20.01, 29.77, 39.18, 60.34, 98.21, 122.85, 127.82, 129.92, 130.95, 132.85, 133.66, 148.04, 165.88, 167.07; HRMS (M) Found 273.1370 (Calcd for $C_{16}H_{19}NO_3$ 273.1366). Anal. Calcd for $C_{16}H_{19}NO_3$: C 70.31; H 7.01; N 5.12. Found: C 70.26; H 7.18; N 5.17. Z isomer (**2.74Z**) (from mixture): ^1H NMR ($CDCl_3$) δ 5.88 (s, =CH); ^{13}C NMR ($CDCl_3$) δ 13.76, 19.71, 30.93, 41.84, 60.44, 93.84, 119.77, 123.38, 128.10, 130.59, 132.31, 137.75, 143.86, 164.71, 168.56.

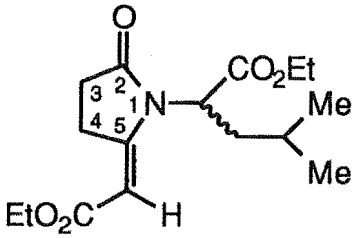
General method B with hydroxy lactam (**2.49**) (14mg, 0.048mmol) and a catalytic quantity of PTSA, in benzene (15mL), and a reflux time of 3h gave a mixture of E and Z isomers (**2.74E** and **2.74Z**, respectively) (95% E : 5% Z by ^1H NMR): Yield 9mg, oil, 23%; ^1H NMR ($CDCl_3$) as given above.

(1'R,S) (E)-1-(1-Ethoxycarbonyleth-1-yl)-5-ethoxycarbonylmethylidene-2-pyrrolidinone (2.68):



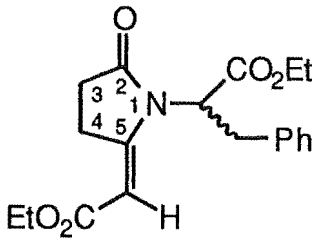
General method A with keto-amide (**2.42**) (82mg, 0.29mmol) and a catalytic quantity of PTSA, in 1, 2-dichloroethane (15mL), and a reflux time of 24h: Yield 65mg, oil, 84%; bp 120 °C (1mm); IR (film) 1740, 1710 and 1630cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25 (t, J=7.1Hz, CH_2CH_3), 1.27 (t, J=7.1Hz, CH_2CH_3), 1.52 (d, J=7.3Hz, $NCHCH_3$), 2.59 (t, J=7.5Hz, $(H\text{-}3)_2$), 3.28 (m, $(H\text{-}4)_2$), 4.15 (q, J=7.1Hz, $=CHCO_2CH_2$), 4.21 (m, $NCHCO_2CH_2$), 4.89 (q, J=7.3Hz, NCH), 5.10 (t, J=2.0Hz, =CH); ^{13}C NMR ($CDCl_3$) δ 13.10, 13.98, 14.29, 24.65, 27.60, 49.18, 59.50, 61.77, 92.43, 157.76, 166.93, 169.14, 176.17; HRMS (M) Found 269.1261 (Calcd for $C_{13}H_{19}NO_5$ 269.1264). Anal. Calcd for $C_{13}H_{19}NO_5$: C 57.98; H 7.11; N 5.20. Found: C 57.86; H 6.85; N 5.20.

*(1'R,S) (E)-1-(1-Ethoxycarbonyl-4-methylpent-2-yl)-5-ethoxycarbonylmethylidene-2-pyrrolidinone (**2.69**):*



General Method A with keto-amide (**2.43**) (155mg, 0.47 mmol) and a catalytic quantity of PTSA, in 1, 2-dichloroethane (15mL), and a reflux time of 20h: Yield 95mg, oil, 84%; bp 155 °C (1mm); IR (film) 1750, 1715 and 1630cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.92 (d, J=4.6Hz, CHC**H$_3$**), 0.94 (d, J=4.5Hz, CHC**H$_3$**), 1.24 (t, J=7.0Hz, CH_2C**H$_3$**), 1.27 (t, J=7.2Hz, CH_2C**H$_3$**), 1.46 (m, C**H**$(CH_3)_2$), 1.95 (t, J=7.2Hz, NCHC**H$_2$**), 2.60 (m, (**H-3**)$_2$), 3.27 (m, (**H-4**)$_2$), 4.14 (q, J=7.2Hz, =CHCO$_2$C**H$_2$**), 4.21 (m, NCHCO$_2$C**H$_2$**), 4.97 (t, J=7.5Hz, NC**H**), 5.10 (t, J=1.9Hz, =C**H**); ^{13}C NMR ($CDCl_3$) δ 13.96, 14.24, 21.54, 22.84, 24.48, 25.18, 27.56, 35.77, 52.05, 59.47, 61.67, 92.83, 157.94, 166.93, 169.17, 176.54; HRMS (M) Found 311.1736 (Calcd for $C_{16}H_{25}NO_5$ 311.1734). Anal. Calcd for $C_{16}H_{25}NO_5$: C 61.72; H 8.09; N 4.50. Found C 61.61; H 8.23; N 4.65.

*(1'R,S) (E)-1-(1-Ethoxycarbonyl-2-phenyleth-1-yl)-5-ethoxycarbonylmethylidene-2-pyrrolidinone (**2.70**):*

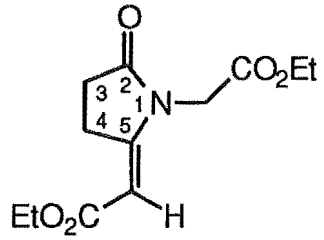


General method A with keto-amide (**2.44**) (165mg, 0.45mmol) and a catalytic quantity of PTSA, in 1, 2-dichloroethane (15mL), and a reflux time of 24h: Yield 140mg, oil, 86%; bp 200 °C (1mm); IR (film) 1745, 1710 and 1630cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.26 (t, J=7.1Hz, C**H$_3$**), 1.28 (t, J=7.1Hz, C**H$_3$**), 2.29 (m, 1H, (**H-3**)$_a$), 2.46 (m, 1H, (**H-3**)$_b$), 3.13 (m, (**H-4**)$_2$), 3.29 (dd, J=10.8, 14.2Hz, 1H, C**H$_a$**Ph), 3.45 (dd, J=5.5, 14.1Hz, 1H, C**H$_b$**Ph), 4.15 (q, J=7.1Hz, =CHCO$_2$C**H$_2$**), 4.25 (m, NCHCO$_2$C**H$_2$**), 5.09 (t, J=8.0Hz, NC**H**), 5.11 (t, J=1.9Hz, =C**H**), 7.13 (m, (**Ph**)$_2$), 7.24 (m, (**Ph**)$_3$); ^{13}C NMR ($CDCl_3$) δ 13.95, 14.24, 24.40, 27.17, 32.90, 54.88, 59.45, 61.86, 92.69, 126.85, 128.32, 128.81, 136.17, 158.02, 166.85, 168.36, 176.18; HRMS (M) Found 345.1579 (Calcd for $C_{19}H_{23}NO_5$

345.1577). Anal. Calcd for $C_{19}H_{23}NO_5$: C 66.07; H 6.71; N 4.06. Found: C 65.99; H 6.76; N 4.23.

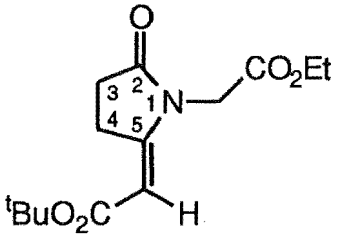
General Method B with keto-amide (**2.44**) (18mg, 0.050mmol) and a catalytic quantity of PTSA, in benzene (15mL), and a reflux time of 6h gave comparable results: Yield 4mg, oil, 23%; ^{1}H NMR ($CDCl_3$) as given above.

*(E)-1 Ethoxycarbonylmethyl-5-ethoxycarbonylmethylidene-2-pyrrolidinone (**2.71**):*



General method B with keto-amide (**2.45**) (40mg, 0.15mmol) and a catalytic quantity of PTSA, in benzene (15mL), and a reflux time of 2h: Yield 31mg, white solid, 83%; mp 111-112 °C (ethyl acetate/petroleum ether, colourless crystals); IR (KBr) 1760, 1720 and 1660cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, J=7.1Hz, C**H**$_3$), 1.29 (t, J=7.1Hz, C**H**$_3$), 2.64 (m, (**H-3**)$_2$), 3.31 (m, (**H-4**)$_2$), 4.16 (q, J=7.1Hz, OC**H**$_2$), 4.23 (q, J=7.1Hz, OC**H**$_2$), 4.28 (s, NC**H**$_2$), 5.05 (t, J=2.0Hz, =C**H**); ^{13}C NMR ($CDCl_3$) δ 14.05, 14.35, 24.74, 27.76, 41.69, 59.65, 61.92, 92.10, 158.97, 166.49, 166.89, 176.47; HRMS (M) Found 255.1112 (Calcd for $C_{12}H_{17}NO_5$ 255.1107). Anal. Calcd for $C_{12}H_{17}NO_5$: C 56.46; H 6.71; N 5.49. Found: C 56.56; H 6.85; N 5.26.

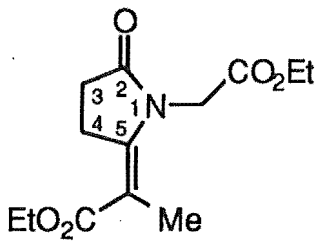
*(E)-1-Ethoxycarbonylmethyl-5-(tert-butoxycarbonyl)methylidene-2-pyrrolidinone (**2.72**):*



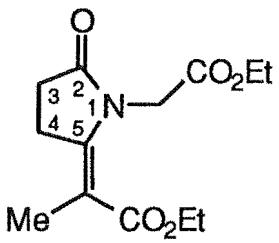
Keto-amide (**2.46**) (33mg, 0.11mmol) was absorbed onto a 1mm silica gel chromatotron plate. Elution with a gradient of ethyl acetate (5-30%) in petroleum ether yielded enamino ester (**2.72**) as a white solid (7mg, 23%): mp 98-99.5 °C; IR (KBr) 1760, 1740, 1710 and 1640cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.30 (t, J=7.1Hz, CH_2C**H**$_3$), 1.48 (s, C(C**H**$_3$)$_3$), 2.62 (m, (**H-3**)$_2$),

3.28 (m, (**H-4**)$_2$), 4.23 (q, J=7.1Hz, OC**H**$_2$), 4.26 (s, NC**H**$_2$), 4.98 (t, J=2.0Hz, =C**H**); ^{13}C NMR ($CDCl_3$) δ 14.02, 24.62, 27.77, 28.30, 41.65, 61.84, 79.78, 94.04, 157.74, 166.33, 166.60, 176.44; HRMS (M) Found 283.1415 (Calcd for $C_{14}H_{21}NO_5$ 283.1420). Anal. Calcd for $C_{14}H_{21}NO_5$: C 59.35; H 7.47; N 4.94. Found: C 58.97; H 7.42; N 5.06.

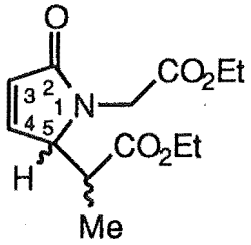
*(E)- and (Z)-1-Ethoxycarbonylmethyl-5-(1-ethoxycarbonylethylidene)-2-pyrrolidinone (**2.73E** and **2.73Z**) and (5R,S) and (5'R,S) 5-(Ethoxycarbonyleth-1-yl)-1-ethoxycarbonylmethyl-pyrrolid-3-en-2-one (**2.80**):*



2.73E



2.73Z



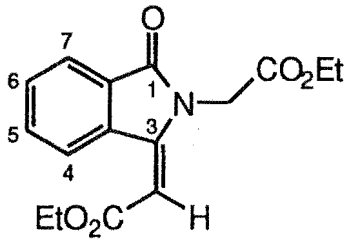
2.80

General method A with keto-amide (**2.47**) (116mg, 0.40mmol) and a catalytic quantity of PTSA, in 1, 2-dichloroethane (15mL), and a reflux time of 10h yielded E- and Z-enamino esters (**2.73E** and **2.73Z**, respectively) in the ratio 74% E : 26% Z, by ^{1}H NMR. Purification by radial chromatography using a 1mm silica gel chromatotron plate, eluting with 80% petroleum ether/20% ethyl acetate gave E-enamino ester (**2.73E**) as an oil: 46mg, 42%; unstable to distillation; IR (film) 1750, 1710 and 1630cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.29 (t, J=7.1Hz, CH_2CH_3), 1.30 (t, J=7.1Hz , CH_2CH_3), 1.97 (t, J=1.4Hz, =CCH_3), 2.56 (m, (**H-3**)$_2$), 3.22 (m, (**H-4**)$_2$), 4.18 (q, J=7.1Hz, OC**H**$_2$), 4.24 (q, J=7.1Hz, OC**H**$_2$), 4.57 (s, NC**H**$_2$); ^{13}C NMR ($CDCl_3$) δ 13.29, 14.09, 14.32, 27.89, 28.28, 45.07, 60.27, 61.80, 101.93, 152.68, 168.21, 168.80, 178.37; HRMS (M) Found 269.1265 (Calcd for $C_{13}H_{19}NO_5$ 269.1264). Further elution gave Z-enamino ester (**2.73Z**) as an oil: 16mg, 15%; IR (film) 1750, 1710 and 1630cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.26 (t, J=7.1Hz, CH_2CH_3), 1.28 (t, J=7.1Hz, CH_2CH_3), 1.89 (t, J=1.0Hz, =CCH_3), 2.59 (m, (**H-3**)$_2$), 2.77 (m, (**H-4**)$_2$), 4.16 (q, J=7.1Hz, OC**H**$_2$), 4.17 (q, J=7.1Hz, OC**H**$_2$), 4.56 (s, NC**H**$_2$); ^{13}C NMR ($CDCl_3$) δ 14.10, 14.20, 16.11, 25.98, 27.69, 44.70, 60.66, 61.27, 101.61, 146.14, 167.89, 168.16, 177.76; HRMS (M) Found 269.1266 (Calcd for $C_{13}H_{19}NO_5$ 269.1264). Further elution gave the endocyclic isomer (**2.80**) as 1 : 1 mixture of two diastereoisomers: 8mg, oil, 7%; ^{1}H NMR ($CDCl_3$) δ 0.96 (d, J=7.3Hz, CHC**H**$_3$), 1.14 (d, J=7.1Hz, CHC**H**$_3$), 1.22 (t, J=7.2Hz, CH_2CH_3), 1.29 (m, 9H, 3x

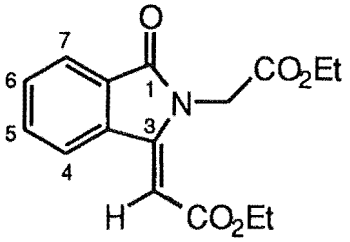
CH_2CH_3), 2.86 (m, $CHCH_3$), 2.94 (m, $CHCH_3$), 3.72 (AB_q, J_{AB}= 17.9Hz, 1H, NCH_a), 3.74 (AB_q, J_{AB}=18.0Hz, 1H, NCH_a), 4.07-4.25 (m, 8H, 4x OCH_2), 4.56 (AB_q, J_{AB}= 17.9Hz, 1H, NCH_b), 4.71 (AB_q, J_{AB}=18.0Hz, 1H, NCH_b), 4.72 (m, **H-5**), 4.77 (m, **H-5**), 6.25 (m, 2H, 2x **H-3**), 7.10 (dd, J=1.8, 6.0Hz, **H-4**), 7.15 (dd, J=1.6, 6.1Hz, **H-4**); HRMS (M) Found 269.1258 (Calcd for $C_{13}H_{19}NO_5$ 269.1290). Over 3 days at 20 °C the relative proportion of one diastereoisomer (the one for which the ^1H δ values are underlined above) increased as the other began to form E- and Z-enamino esters (**2.73E** and **2.73Z**, respectively): ^{13}C NMR ($CDCl_3$) major diastereoisomer of (**2.80**) δ 10.49, 14.11, 14.17, 39.19, 41.65, 61.24, 61.50, 63.38, 128.10, 146.63, 168.85, 171.94, 173.21.

General method B with keto-amide (**2.47**) (71mg, 0.25mmol) and a catalytic quantity of PTSA, in benzene (15mL), and a reflux time of 6h gave E- and Z-enamino esters (**2.73E** and **2.73Z**, respectively) in the ratio 86% E : 14% Z, by ^1H NMR. Purification by radial chromatography as above gave E- and Z-enamino esters (**2.73E** and **2.73Z**, respectively): 15mg, oil, 15%; ^1H NMR ($CDCl_3$) as given above, and endocyclic isomer (**2.80**) as a 1 : 1 mixture of diastereoisomers: 30mg, oil, 45%; ^1H NMR ($CDCl_3$) as given above.

(E)- and (Z)-2-ethoxycarbonylmethyl-3-ethoxycarbonylmethylidene-isoindolone (**2.75E** and **2.75Z**):



2.75E



2.75Z

General method A with hydroxy lactam (**2.50**) (134mg, 0.42mmol) and a catalytic quantity of PTSA, in 1, 2-dichloroethane (15mL), and a reflux time of 3h gave a mixture of E- and Z-enamino esters (**2.75E** and **2.75Z**, respectively) (60% E : 40% Z, by ^1H NMR): Yield 114mg, white solid, 90%. On recrystallization (ethanol/H_2O) the isomer ratio changed to 45% E : 55% Z by ^1H NMR: IR (KBr) 1750, 1730 and 1650cm^{-1}; ^1H NMR ($CDCl_3$) E isomer (**2.75E**) from mixture δ 1.29 (t, J=7.1Hz, CH_3), 1.35 (t, J=7.1Hz, CH_3), 4.25 (q, J=7.1Hz, OCH_2), 4.27 (q, J=7.1Hz, OCH_2), 4.56 (s, NCH_2), 5.54 (s, =CH), 7.61 (dt, J=1.1, 7.4Hz, **H-6**), 7.70 (dt, J=1.4, 7.7Hz, **H-5**), 7.89

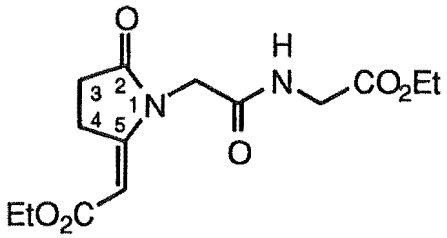
(dd, J=0.9, 6.7Hz, **H-7**), 9.10 (d, J=7.8Hz, **H-4**); Z isomer (**2.75Z**) from mixture δ 1.28 (t, J=7.1Hz, CH_3), 1.31 (t, J=7.1Hz, CH_3), 4.19 (q, J=7.1Hz, OCH_2), 4.21 (q, J=7.1Hz, OCH_2), 5.15 (s, NCH_2), 5.92 (s, =CH), 7.56-7.73 and 7.86-7.89 (m, 4H, **H-4**, **H-5**, **H-6** and **H-7**); ^{13}C NMR ($CDCl_3$) E isomer (**2.75E**) from mixture δ 13.97, 14.16, 41.00, 60.44, 61.75, 98.68, 123.27, 128.14, 129.44, 131.21, 133.35, 133.74, 147.71, 165.45, 167.16, 168.74, Z isomer (**2.75Z**) from mixture δ 14.05, 14.08, 44.39, 60.21, 61.11, 94.56, 120.14, 123.76, 127.43, 130.90, 132.84, 137.72, 144.44, 164.83, 166.73, 168.23; HRMS (M) Found 303.1116 (Calcd for $C_{16}H_{17}NO_5$ 303.1107). Anal. Calcd for $C_{16}H_{17}NO_5$: C 63.36; H 5.65; N 4.62. Found: C 63.36; H 5.65; N 4.74.

Isomerization of Z-Enamino Ester (**2.75Z**): E- and Z-enamino esters (**2.75E** and **2.75Z**, respectively) (4mg) from above (ie; 60% E : 40% Z) and PTSA (1 crystal) were dissolved in $CDCl_3$ (0.7mL) and heated at 60 °C. After 3h the isomer ratio was 70% E : 30% Z, by ^1H NMR, and this ratio had not changed after heating for a further 20h.

SECTION E.2.3

EXTENSION OF THE PEPTIDE CHAIN

*Preparation of (E)-5-Ethoxycarbonylmethylidene-1-(N-ethoxycarbonylmethyl)-carbamoylmethyl-2-pyrrolidinone (**2.93**):*

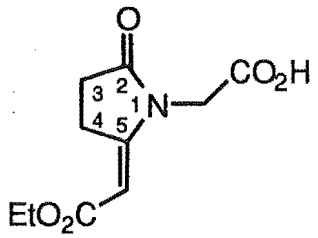


METHOD A: Glycylglycine ethylester hydrochloride (69mg, 0.35mmol, 1.8equiv) and triethylamine (46μL, 0.35mmol, 1.8equiv) were added to enollactone[E.05] (**2.33a**) (33mg, 0.19mmol, 1equiv), dissolved in 1, 2-dichloroethane (30mL), and the mixture was refluxed for 16h with azeotropic removal of H_2O. The solvent was evaporated and purification of the residue by radial chromatography using a 1mm silica gel chromatotron plate, eluting with 50% ethyl acetate/50% CH_2Cl_2 yielded enamino ester (**2.93**) as a white solid (48mg, 79%): mp 131-133 °C (ethyl acetate/petroleum ether); FTIR (KBr) 3298, 1763, 1742, 1705, 1664, 1619 and 1560cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.27 (t, J=7.1Hz, C**H**$_3$), 1.29 (t, J=7.1Hz, C**H**$_3$), 2.66 (m, (**H-3**)$_2$), 3.32 (m, (**H-4**)$_2$), 4.03 (d, J=4.9Hz, NHC**H**$_2$), 4.15 (q, J=7.1Hz, OC**H**$_2$), 4.23 (q, J=7.1Hz, OC**H**$_2$), 4.25 (s, NC**H**$_2$), 5.25 (t, J=2.0Hz, =C**H**), 6.27 (bs, N**H**); ^{13}C NMR ($CDCl_3$) δ 14.06, 14.32, 24.73, 27.80, 41.38, 43.68, 59.68, 61.71, 92.90, 158.75, 165.72, 166.96, 169.38, 176.97; HRMS (M) Found 312.1319 (Calcd for $C_{14}H_{20}N_2O_6$ 312.1321). Anal. Calcd for $C_{14}H_{20}N_2O_6$: C 53.84; H 6.45; N 8.97. Found: C 53.70; H 6.42; N 8.72.

METHOD B: DCC (1,3-dicyclohexylcarbodiimide) (40mg, 0.19mmol, 1equiv), glycine ethylester hydrochloride (30mg, 0.21mmol, 1.1equiv) and triethylamine (28μL, 0.21mmol, 1.1equiv) were added to enamino ester (**2.95**) (0.19mmol, 1equiv) dissolved in CH_2Cl_2 (5mL) and the mixture was stirred at 20 °C for 16h. The resulting solution was washed with H_2O (5mL), dried ($MgSO_4$) and the solvent evaporated. Purification by radial chromatography using a 1mm silica gel chromatotron plate, eluting with 50% ethyl

acetate/50% CH_2Cl_2 yielded enamino ester (**2.93**) (28mg, 40%) as a white solid; ^1H NMR ($CDCl_3$) as given above.

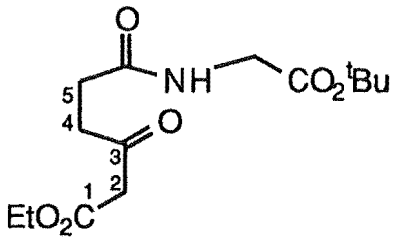
*Preparation of (E)-1-Carboxymethyl-5-ethoxycarbonylmethylidene-2-pyrrolidinone (**2.95**):*



METHOD A: General method A for the preparation of enamino esters (See page 183) with keto-amide (**2.94**) (59mg, 0.20mmol) and a catalytic quantity of PTSA, in 1, 2-dichloroethane (15mL), and a reflux time of 5h. The acid (**2.95**) is significantly soluble in H_2O, hence after refluxing, the solvent was evaporated and the solid thus obtained was used in subsequent steps without further purification: FTIR (KBr) 1734, 1702 and 1619cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.29 (t, J=7.1Hz, CH_3), 2.65 (m, $(H\text{-}3)_2$), 3.30 (m, $(H\text{-}4)_2$), 4.17 (q, J=7.1Hz, OCH_2), 4.34 (s, NCH_2), 5.11 (t, J=1.8Hz, =CH); ^{13}C NMR ($CDCl_3$) δ 14.33, 24.78, 27.76, 41.36, 60.00, 92.43, 158.91, 167.23, 170.38, 176.87; HRMS (M) Found 227.0790 (Calcd for $C_{10}H_{13}NO_5$ 227.0794).

METHOD B: Enollactone[E.05] (**2.33a**) (20mg, 0.12mmol, 1equiv) and glycine (11mg, 0.14mmol, 1.2equiv), dissolved in a mixture of CH_2Cl_2 (5mL), DMF (5mL) and H_2O (3mL), were stirred at 20 °C for 44h. The solvent was evaporated by boiling at atmospheric pressure and finally at 1mm to give an oil (33mg) which contained enamino ester (**2.95**) and residual DMF: ^1H NMR ($CDCl_3$) as given above. This sample was not used subsequently.

Preparation of Ethyl 5-(N-(1-tert-butoxycarbonylmethyl)carbamoyl)-3-oxopentanoate **(2.94):**

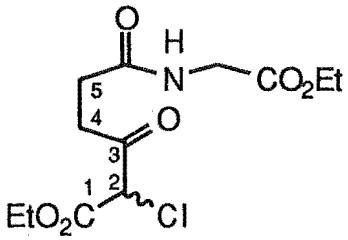


General method for the preparation of amino acid-derived keto-amides (See page 179) with enollactone (**2.33a**) (35mg, 0.21mmol, 1equiv), glycine *tert*-butylester hydrochloride (51mg, 0.31mmol, 1.5equiv) and triethylamine (41µL, 0.31mmol, 1.5equiv), in CH_2Cl_2 (15mL): Yield 63mg, white solid, quant; FTIR (KBr) 3276, 1736, 1713, 1665, 1642 and 1563cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, J=7.1Hz, CH_3), 1.47 (s, $C(CH_3)_3$), 2.55 (t, J=6.6Hz, **(H-4)**$_2$), 2.92 (t, J=6.6Hz, **(H-5)**$_2$), 3.50 (s, **(H-2)**$_2$), 3.92 (d, J=4.9Hz, NCH_2), 4.20 (q, J=7.1Hz, OCH_2), 6.09 (bs, **NH**); ^{13}C NMR ($CDCl_3$) δ 13.98, 27.91, 29.34, 37.71, 42.01, 49.13, 61.31, 82.14, 167.01, 168.98, 171.39, 201.85; HRMS (M-18) 283.1420 (Calcd for $C_{14}H_{21}NO_5$ 283.1420).

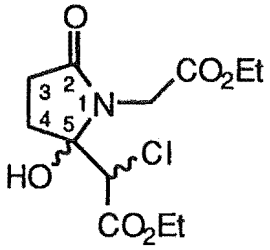
SECTION E.2.4

PREPARATION OF SUCCINIMIDE-BASED CHLORO ENAMINO ESTERS

*Preparation of Ethyl (2R,S) 2-chloro-5-(N-(1-ethoxycarbonylmethyl)carbamoyl)-3-oxopentanoate (**2.96**) and (5R and/or S) and (5'R and/or S) Chloro(ethoxycarbonyl)methyl-1-ethoxycarbonylmethyl-5-hydroxy-2-pyrrolidinone (**2.97**):*



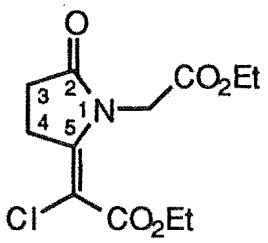
2.96



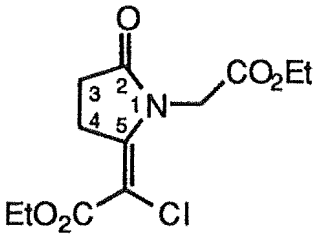
2.97

Glycine ethylester hydrochloride (27mg, 0.19mmol, 1.3equiv) and triethylamine (25μL, 0.19mmol, 1.3equiv) were added to enollactone (**1.11**) (30mg, 0.15mmol, 1equiv), dissolved in ethyl acetate (3mL), and the mixture was stirred for 3h. The solvent was evaporated to give an oil which contained, by ^{1}H NMR, keto-amide (**2.96**) and hydroxy lactam (**2.97**) in the ratio 9 : 1, respectively: ^{1}H NMR ($CDCl_3$) keto-amide (**2.96**) from mixture δ 1.29 (t, J=7.1Hz, CH_3), 1.32 (t, J=7.1Hz, CH_3), 2.60 (t, J=6.5Hz, (**H-5**)$_2$), 3.07 (m, (**H-4**)$_2$), 4.02 (d, J=5.2Hz, NHCH_2), 4.22 (q, J=7.1Hz, OCH_2), 4.30 (q, J=7.1Hz, OCH_2), 4.90 (s, CHCl), 6.10 (bs, NH). Ethyl acetate (3mL) was added to the residue, the mixture was filtered and the solvent was evaporated to give an oil (47mg, quant) which contained, by ^{1}H NMR, keto-amide (**2.96**) and hydroxy lactam (**2.97**) in the ratio 3 : 7, respectively. Hydroxy lactam (**2.97**) was present as a mixture of diastereoisomers, by ^{1}H NMR. ^{1}H NMR ($CDCl_3$) hydroxy lactam (**2.97**) from mixture δ 1.26-1.40 (m, CH_3), 2.17, 2.48, 2.68 and 2.89 (m, **H-3** and **H-4**), 3.71 (AB_q, J_{AB}=18.1Hz, 1H, NCH_a), 4.13 (AB_q, J_{AB}=17.8Hz, 1H, NCH_a), 4.20-4.32 (m, OCH_2), 4.38 (s, CHCl), 4.50 (AB_q, J_{AB}=17.8Hz, 1H, NCH_b), 4.53 (s, CHCl), 4.68 (AB_q, J_{AB}=18.1Hz, 1H, NCH_b).

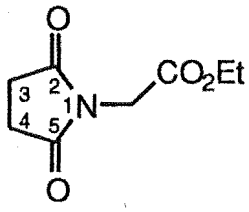
*Preparation of (E)- and (Z)-5-Chloroethoxycarbonylmethylidene-1-ethoxycarbonylmethyl-2-pyrrolidinone (**2.98E** and **2.98Z**) and 1-Ethoxycarbonylmethyl pyrrolidine-2,5-dione*[E.10] *(**2.99**):*



2.98E



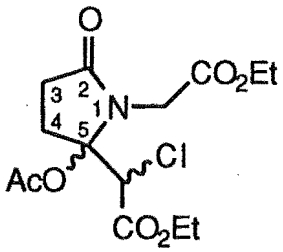
2.98Z



2.99

METHOD A: Keto-amide - hydroxy lactam mixture (**2.96/2.97**) (32mg, 0.10mmol) and a catalytic quantity of PTSA were dissolved in 1, 2-dichloroethane (10mL). Some activated 4Å molecular sieves were added and the mixture was stirred at 70 °C for 6.5 days. The mixture was cooled to 20 °C, filtered and the solvent evaporated. Purification by radial chromatography using a 1mm silica gel chromatotron plate, eluting with 72% petroleum ether/22% ethyl acetate/6% CH_2Cl_2 yielded E-enamino ester (**2.98E**) as an oil (4mg, 13%): IR (film) 3435, 1740, 1720 and 1640cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.27 (t, J=7.2Hz, C**H**$_3$), 1.32 (t, J=7.2Hz, C**H**$_3$), 2.64 (m, (**H-3**)$_2$), 3.00 (m, (**H-4**)$_2$), 4.19 (q, J=7.2Hz, OC**H**$_2$), 4.22 (q, J=7.2Hz, OC**H**$_2$), 4.68 (s, NC**H**$_2$); ^{13}C NMR ($CDCl_3$) δ 14.06, 14.09, 27.19, 28.19, 45.22, 61.46, 61.94, 99.73, 150.21, 162.81, 167.75, 177.79; HRMS (M) Found 291.0702 (Calcd for $C_{12}H_{16}{}^{37}ClNO_5$ 291.0688), Found 289.0718 (Calcd for $C_{12}H_{16}{}^{35}ClNO_5$ 289.0718). Further elution gave Z-enamino ester (**2.98Z**) as an oil (4mg, 13%): IR (film) 3465, 1740, 1690 and 1590cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.29 (t, J=7.1Hz, C**H**$_3$), 1.33 (t, J=7.1Hz, C**H**$_3$), 2.64 (m, (**H-3**)$_2$), 3.35 (m, (**H-4**)$_2$), 4.24 (q, J=7.1Hz, OC**H**$_2$), 4.25 (q, J=7.1Hz, OC**H**$_2$), 4.86 (s, NC**H**$_2$); ^{13}C NMR ($CDCl_3$) δ 14.11, 14.22, 27.76, 27.76, 44.63, 61.46, 61.80, 97.22, 152.26, 163.95, 167.92, 177.42; HRMS (M) Found 291.0640 (Calcd for $C_{12}H_{16}{}^{37}ClNO_5$ 291.0688), Found 289.0714 (Calcd for $C_{12}H_{16}{}^{35}ClNO_5$ 289.0718). Further elution gave imide[E.10] (**2.99**) as an oil (11mg, 57%): mp 65-68 °C (ethyl acetate/petroleum ether, white crystals) (Lit[E.10] 68 °C); IR (KBr) 1740 and 1710cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.29 (t, J=7.1Hz, C**H**$_3$), 2.81 (s, 4H, 2x NCOC**H**$_2$), 4.22 (q, J=7.1Hz, OC**H**$_2$), 4.25 (s, NC**H**$_2$); ^{13}C NMR ($CDCl_3$) δ 14.06, 28.23, 39.56, 61.95, 166.67, 176.29; HRMS (M) Found 185.0686 (Calcd for $C_8H_{11}NO_4$ 185.0688). Anal. Calcd for $C_8H_{11}NO_4$: C 51.89; H 5.99; N 7.56. Found: C 51.77; H 5.87; N 7.55.

METHOD B:

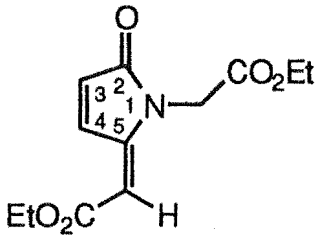


2.100

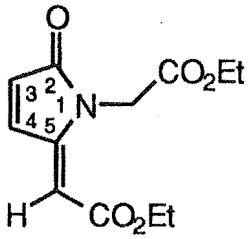
Acetic anhydride (36μL, 0.38mmol, 2equiv) and triethylamine (50μL, 0.38mmol, 2equiv) were added to keto-amide - hydroxy lactam mixture (**2.96/2.97**) (58mg, 0.19mmol, 1equiv) and 4-DMAP (35mg, 0.28mmol, 1.5equiv), dissolved in CH_2Cl_2 (6mL), and the mixture was stirred for 2.5h. The solvent was evaporated and the residue was dissolved in benzene (10mL), washed successively with 0.1N HCl (4x 10mL) and 0.2N NaOH (4x 10mL), dried ($MgSO_4$) and the solvent was evaporated to yield acetate (**2.100**) (43mg, oil, 65%), as a 3 : 2 mixture of two diastereoisomers, by ^1H NMR. This oil was used in subsequent steps without further purification: IR (film) 1750, 1725, 1635 and 1595cm^{-1}; ^1H NMR ($CDCl_3$) both diastereoisomers δ 1.25-1.36 (m, 12H, 4x CH_3), 2.01 (s, major diastereoisomer, $COCH_3$), 2.06 (s, $COCH_3$), 2.40-2.52 (m, 4H, 2x (**H-3**)$_2$), 2.74-2.98 (m, 4H, 2x(**H-4**)$_2$), 3.82-4.32 (m, 12H, 4x OCH_2 and 2x NCH_2), 4.88 (s, major, CHCl), 5.00 (s, CHCl); ^{13}C NMR ($CDCl_3$) δ 13.87, 13.96, 14.05, 21.57, 21.69, 26.69, 27.88, 28.44, 28.61, 41.80, 42.52, 56.67, 58.38, 61.43, 61.49, 62.61, 62.76, 96.04, 96.91, 165.91, 165.77, 166.00, 167.87, 167.97, 168.93, 169.53, 176.39, 176.52; HRMS (M-60) Found 291.0699 (Calcd for $C_{12}H_{16}{}^{37}ClNO_5$ 291.0688), Found 289.0715 (Calcd for $C_{12}H_{16}{}^{35}ClNO_5$ 289.0718).

Acetate (**2.100**) (40mg, 0.11mmol) was dissolved in benzene (5mL) and heated at 65 °C for 90min. The solvent was evaporated to give an oil (25mg) which contained, by ^1H NMR, 85% E- and Z-enamino esters (**2.98E** and **2.98Z**, respectively) (in the ratio of 56% Z : 44% E) and the elimination product (**2.101**) (15%). On distillation (145-160 °C, 1mm) the E- and Z-enamino esters (**2.98E** and **2.98Z**, respectively) formed the elimination product (**2.101**).

*Preparation of (E)- and (Z)-1-Ethoxycarbonylmethyl 5-ethoxycarbonylmethylidene pyrrolid-3-en-2-one (**2.101E** and **2.101Z**):*



2.101E



2.101Z

METHOD A: Keto-amide - hydroxy lactam mixture (**2.96/2.97**) (11mg, 0.036mmol) and a catalytic quantity of PTSA were dissolved in 1, 2-dichloroethane (7mL) and refluxed, with azeotropic removal of H_2O, for 3h. Purification by radial chromatography using a 1mm silica gel chromatotron plate, eluting with 95% CH_2Cl_2/5% ethyl acetate gave an oil which contained E and Z isomers of the elimination product (**2.101E** and **2.101Z**, respectively) in the ratio of 59% E : 41% Z, by ^1H NMR (3.5mg, 39%): ^1H NMR ($CDCl_3$) E isomer (**2.101E**) from mixture δ 1.28 (t, J=7.2Hz, CH_3), 1.32 (t, J=7.2Hz, CH_3), 4.19 (q, J=7.2Hz, OCH_2), 4.22 (q, J=7.2Hz, OCH_2), 4.35 (s, NCH_2), 5.44 (d, J=1.0Hz, =CH), 6.40 (dd, J=1.5, 6.0Hz, **H-3**), 8.21 (d, J=6.0Hz, **H-4**); Z isomer (**2.101Z**) from mixture δ 1.27 (t, J=7.2Hz, CH_3), 1.28 (t, J=7.2Hz, CH_3), 4.17 (q, J=7.2Hz, OCH_2), 4.24 (q, J=7.2Hz, OCH_2), 4.94 (s, NCH_2), 5.44 (s, =CH), 6.36 (d, J=5.7Hz, **H-3**), 6.99 (d, J=5.7Hz, **H-4**); ^{13}C NMR ($CDCl_3$) E isomer (**2.101E**) from mixture δ 14.09, 14.24, 40.56, 60.74, 61.88, 99.69, 126.73, 136.43, 150.48, 165.24, 167.34, 169.79; HRMS (M) Found 253.0953 (Calcd for $C_{12}H_{15}NO_5$ 253.0951).

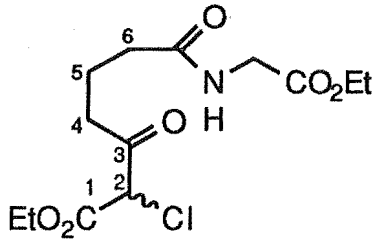
METHOD B: E- and Z-chloro enamino esters (**2.98E** and **2.98Z**, respectively) (2mg) present in the ratio of 1 E : 1 Z, dissolved in $CDCl_3$ (0.7mL) containing PTSA (1 crystal), were heated at 55 °C and the reaction was monitored by ^1H NMR. After 15min all the E isomer (**2.98E**) had been converted to the elimination products (**2.101E** and **2.101Z**) and after 45min all the Z isomer (**2.98Z**) had been converted to the elimination products (**2.101E** and **2.101Z**) to give an isomer ratio of 80% E : 20% Z, respectively, by ^1H NMR.

METHOD C: The elimination products (**2.101E** and **2.101Z**) formed from chloro enamino esters (**2.98E** and **2.98Z**) on silica gel chromatotron plates during radial chromatography. The extent of conversion and the E/Z isomer ratio varied depending on the time spent on the silica.

SECTION E.2.5

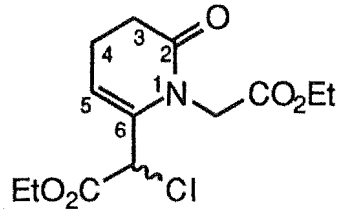
ATTEMPTED PREPARATION OF GLUTARIMIDE-BASED CHLORO ENAMINO ESTERS

Ethyl (2R,S) 2-chloro-6-(N-(1-ethoxycarbonylmethyl)carbamoyl)-3-oxohexanoate **(2.103):**

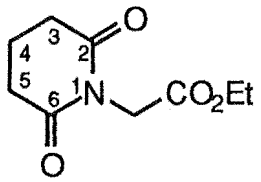


General method for the preparation of amino acid derived keto-amides (See page 179) with enollactone (**1.12**) (38mg, 0.17mmol, 1equiv), glycine ethylester hydrochloride (34mg, 0.24mmol, 1.4equiv) and triethylamine (32μL, 0.24mmol, 1.4equiv), in CH_2Cl_2 (5mL): Yield 47mg, pale yellow oil, 84%; IR (film) 3350, 1760, 1670, and 1550cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.29 (t, J=7.1Hz, C**H**$_3$), 1.32 (t, J=7.1Hz, C**H**$_3$), 2.01 (m, (**H-5**)$_2$), 2.30 (t, J=7.3Hz, (**H-6**)$_2$), 2.84 (dt, J=1.5, 6.8Hz, (**H-4**)$_2$), 4.02 (d, J=5.2Hz, NC**H**$_2$), 4.22 (q, J=7.1Hz, OC**H**$_2$), 4.29 (q, J=7.1Hz, OC**H**$_2$), 4.81 (s, C**H**Cl), 6.01 (bs, N**H**); ^{13}C NMR ($CDCl_3$) δ 13.86, 14.06, 19.23, 34.38, 37.83, 41.26, 60.71, 61.44, 63.11, 165.04, 169.93, 172.26, 198.72; HRMS (M) Found 323.0952 (Calcd for $C_{13}H_{20}{}^{37}ClNO_6$ 323.0950), Found 321.0984 (Calcd for $C_{13}H_{20}{}^{35}ClNO_6$ 321.0980).

(6'R,S) 6-chloro(ethoxycarbonyl)methyl-1-ethoxycarbonylmethyl-piperid-5-en-6-one **(2.104):**



2.104



2.105

General method A for the preparation of enamino esters (See page 183) with keto-amide (**2.103**) (30mg, 0.093mmol) and a catalytic quantity of PTSA, in 1, 2-dichloroethane (10mL), and reflux time of 6h. Purification by radial chromatography using a 1mm silica gel chromatotron plate, eluting with 60% petroleum ether/40% ethyl acetate gave the

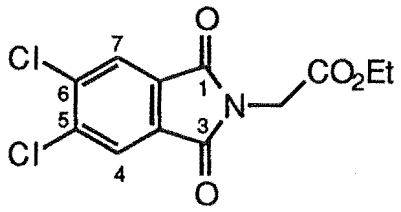
endocyclic isomer (**2.104**) as an oil: Yield 10mg, 38%; bp 155 °C (1mm); IR (film) 3385, 1750, 1685 and 1615cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, J=7.1Hz, C**H$_3$**), 1.31 (t, J=7.1Hz, C**H$_3$**), 2.39 (m, (**H-4**)$_2$), 2.56 (t, J=7.4Hz, (**H-3**)$_2$), 4.20 (q, J=7.1Hz, $CH_2CO_2C\mathbf{H_2}CH_3$), 4.27 (m, $CHClCO_2C\mathbf{H_2}$), 4.41 (AB_q, J_{AB}=18.0Hz, 1H, NC**H$_a$**), 4.61 (AB_q, J_{AB}=18.0Hz, 1H, NC**H$_b$**), 4.97 (s, C**H**Cl), 5.62 (t, J=4.9Hz, **H-5**); ^{13}C NMR ($CDCl_3$) δ 13.92, 14.08, 19.51, 30.60, 43.59, 56.79, 61.42, 63.20, 113.16, 135.36, 166.50, 168.76, 170.98; HRMS (M) Found 305.0845 (Calcd for $C_{13}H_{18}{}^{37}ClNO_5$ 305.0844); Found 303.0857 (Calcd for $C_{13}H_{18}{}^{35}ClNO_5$ 303.0874). Anal. Calcd for $C_{13}H_{18}ClNO_5$: C 51.41; H 5.97; N 4.61. Found: C 51.11; H 5.97; N 4.71. Further elution gave a fraction (4mg) containing recovered keto-amide (**2.103**) and imide[E.10] (**2.105**) in a ratio of 5 : 4, by ^{1}H NMR.

SECTION E.2.6

ATTEMPTED PREPARATION OF PHTHALIMIDE-BASED HALO ENAMINO ESTERS

Reaction with E-dichlorophthalic bromo enollactone (**1.17E**):

Preparation of 2-Ethoxycarbonylmethyl-5,6-dichloro-isoindoline-1,3-dione (**2.110**):



2.110

Glycine ethylester hydrochloride (24mg, 0.17mmol, 1.3equiv) and triethylamine (23μL, 0.17mmol, 1.3equiv) were added to E-dichlorophthalic bromo enollactone (**1.17E**) (50mg, 0.14mmol, 1equiv), dissolved in CH_2Cl_2 (10mL). The mixture was stirred at 20 °C for 16h, washed with H_2O (10mL) and the solvent evaporated to give an intractable mixture (67mg, oil).

This oil (67mg) and a catalytic quantity of PTSA were dissolved in 1, 2-dichloroethane (10mL) and activated 4Å molecular sieves were added. The mixture was stirred at 70 °C for 6 days. The solvent was evaporated and the residue was purified by radial chromatography using a 1mm silica gel chromatotron plate, eluting with a gradient of CH_2Cl_2 (45-80%) in petroleum ether to give imide (**2.110**) as a white solid (20mg, 49%): mp 118-120 °C (petroleum ether, white crystals): IR (KBr) 1760 and 1720cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.29 (t, J=7.1Hz, C**H$_3$**), 4.23 (q, J=7.1Hz, OC**H$_2$**), 4.42 (s, NC**H$_2$**), 7.97 (s, (**Ph**)$_2$); ^{13}C NMR ($CDCl_3$) δ 14.09, 39.24, 62.09, 125.72, 131.10, 139.28, 165.52, 166.79; HRMS (M) Found 302.9879 (Calcd for $C_{12}H_9{}^{37}Cl^{35}ClNO_4$ 302.9880). Anal. Calcd for $C_{12}H_9Cl_2NO_4$: C 47.71; H 3.00; N 4.64. Found: C 48.01; H 2.99; N 4.63.

Reaction with E-dichlorophthalic chloro enollactone (**1.15E**):

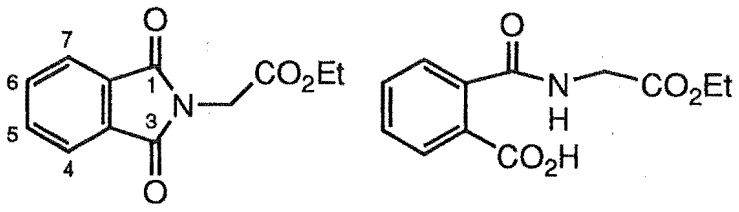
Glycine ethylester hydrochloride (12mg, 0.087mmol, 1.4equiv) and triethylamine (12μL, 0.087mmol, 1.4equiv) were added to E-dichlorophthalic chloro enollactone (**1.15E**), dissolved in ethyl acetate (5mL). The mixture was stirred at 20 °C for 16h, filtered and the

solvent was evaporated to give an intractable mixture (21mg, oil) which contained some imide (**2.110**), by ^{1}H NMR: ^{1}H NMR ($CDCl_3$) as given above.

Reaction with Z-phthalic chloro enollactone (**1.14Z**):

Glycine ethylester hydrochloride (7mg, 0.050mmol, 1.4equiv) and triethylamine (17μL, 0.050mmol, 1.4equiv) were added to Z-phthalic chloro enollactone (**1.14Z**) (9mg, 0.036mmol, 1equiv), dissolved in ethyl acetate (3mL). The mixture was stirred at 20 °C for 16h, filtered and the solvent was evaporated to give an intractable mixture (11mg, oil). This oil (11mg) and a catalytic amount of PTSA were dissolved in 1, 2-dichloroethane (5mL) and refluxed, with azeotropic removal of H_2O, for 3h. The solvent was evaporated to give an intractable mixture which contained some imide[E.10] (**2.111**), by ^{1}H NMR (See below for data).

Preparation of 2-Ethoxycarbonylmethyl isoindoline-1,3-dione[E.10] (**2.111**):



2.111 **2.113**

Glycine ethylester hydrochloride (104mg, 0.74mmol, 1.1equiv) and triethylamine (98μL, 0.74mmol, 1.1equiv) were added to phthalic anhydride (**2.112**) (100mg, 0.68mmol, 1equiv), dissolved in ethyl acetate (15mL). The mixture was stirred at 20 °C for 16h, filtered and the solvent was evaporated to give acid-amide (**2.113**) as an oil (171mg, quant): IR (film) 3360, 1720 and 1650cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.31 (t, J=7.2Hz, C**H**$_3$), 4.25 (d, J=4.6Hz, NHC**H**$_2$), 4.25 (q, J=7.2Hz, OC**H**$_2$), 6.77 (bt, J=4.6Hz, N**H**), 7.57 (m, (**Ph**)$_3$), 8.06 (m, (**Ph**)$_1$); ^{13}C NMR ($CDCl_3$) δ 13.87, 41.79, 61.36, 127.85, 129.20, 129.82, 130.67, 131.96, 136.59, 168.92, 169.67, 170.41; HRMS (M-18) Found 233.0693 (Calcd for $C_{12}H_{11}NO_4$ 233.0688).

Acid-amide (**2.113**) (114mg, 0.45mmol) and a catalytic quantity of PTSA were dissolved in 1, 2-dichloroethane (20mL) and refluxed, with azeotropic removal of H_2O, for 8 days. The solvent was evaporated and the residue was purified by radial chromatography using a 1mm silica gel chromatotron plate, eluting with a gradient of

ethyl acetate (0-50%) in CH_2Cl_2. Imide[E,10] (**2.111**) was obtained as a white solid (50mg, 48%): mp 112-114 °C (petroleum ether, white crystals) (Lit[E,10] 114-115 °C): IR (KBr) 1750 and 1730cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, J=7.1Hz, C**H**$_3$), 4.23 (q, J=7.1Hz, OC**H**$_2$), 4.43 (s, NC**H**$_2$), 7.75 (m, (**Ph**)$_2$), 7.89 (m, (**Ph**)$_2$); ^{13}C NMR ($CDCl_3$) δ 14.03, 38.87, 61.82, 123.52, 131.95, 134.15, 167.17, 167.40; HRMS (M) Found 233.0692 (Calcd for $C_{12}H_{11}NO_4$ 233.0688). Anal. Calcd for $C_{12}H_{11}NO_4$: C 61.80; H 4.75; N 6.01. Found: C 62.07; H 4.69; N 6.01.

More Reactions with Dichlorophthalic Bromo Enollactone (**1.17**):

Glycine ethylester hydrochloride (111mg, 0.79mmol, 1.3equiv) and triethylamine (105μL, 0.79mmol, 1.3equiv) were added to E- and Z-dichlorophthalic bromo enollactones (**1.17E** and **1.17Z**) (223mg, 0.61mmol, 1equiv), dissolved in ethyl acetate (8mL), and the mixture was stirred at 20 °C. After 50min 4-DMAP (112mg, 0.91mmol, 1.5equiv), triethylamine (161μL, 1.22mmol, 2equiv) and acetic anhydride (115μL, 1.22mmol, 2equiv) were added and the mixture was stirred for 2h, then filtered. The filtrate was diluted with ethyl acetate (15mL), washed with 10% citric acid solution (20mL) and H_2O (2x 20mL), dried ($MgSO_4$) and the solvent evaporated to give an intractable mixture (176mg, oil) which contained some imide (**2.110**): ^{1}H NMR ($CDCl_3$) as given above.

Oil (5mg) was dissolved in C_6D_6 (0.7mL) and heated at 70 °C. After 1 day the composition, by ^{1}H NMR, had not changed.

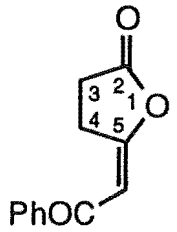
Oil (20mg) was dissolved in toluene (5mL) and refluxed for 16h. At this stage the composition, by ^{1}H NMR, had changed very little. Purification by radial chromatography, using a 1mm silica gel chromatotron plate, eluting with a gradient of ethyl acetate (5-100%) in petroleum ether gave a series of intractable fractions.

Oil (151mg) was heated at 180 °C at 1mm for 2h. Purification by radial chromatography, using a 1mm silica gel chromatotron plate, eluting with a gradient of ethyl acetate (5-100%) in petroleum ether gave a series of intractable fractions.

SECTION E.2.7

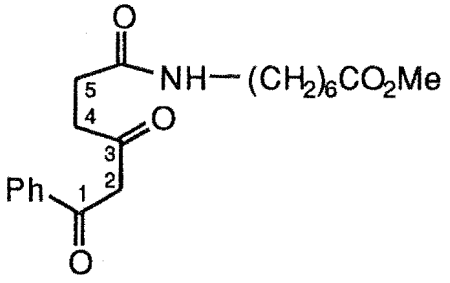
A SYNTHETIC INTERMEDIATE OF PROSTAGLANDIN ANALOGUES

*Preparation of (E)-5-(2-oxo-2-phenylethylidene)-2-tetrahydrofuranone (**2.115**):*

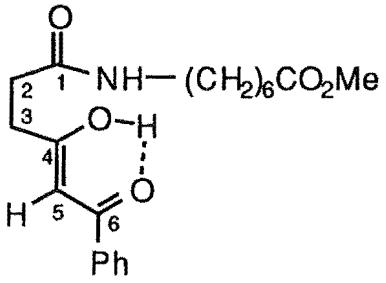


Succinic anhydride (0.84g, 0.0084mol, 1equiv) and $Ph_3P{=}CHCOPh$[E.11] (4.77g, 0.013mol, 1.5equiv) were dissolved in CH_2Cl_2 (80mL) and refluxed for 3 months, at which time more $Ph_3P{=}CHCOPh$ (1.84g, 0.0048mol, 0.6equiv) was added and the reflux continued. After another month, more $Ph_3P{=}CHCOPh$ (0.65g, 0.0017mol, 0.2equiv) was added and the reflux continued for one month more. The solvent was evaporated and the residue was purified by silica column chromatography, eluting with CH_2Cl_2 to give enollactone (**2.115**) as a beige solid (1.68g, 99%): mp 156-159 °C (CH_2Cl_2/petroleum ether, cream crystals); FTIR (KBr) 1802, 1696 and 1668cm^{-1}; ^1H NMR (d_6-acetone) δ 2.99 (m, (**H-3**)$_2$), 3.64 (m, (**H-4**)$_2$), 7.06 (t, J=2.1Hz, =C**H**), 7.65 (m, (**Ph**)$_2$), 7.73 (m, (**Ph**)$_1$), 8.13 (m, (**Ph**)$_2$); ^{13}C NMR (d_6-acetone) δ 25.77, 27.77, 99.51, 127.94, 128.87, 132.78, 139.07, 171.18, 174.43, 189.59; HRMS (M) Found 202.0632 (Calcd for $C_{12}H_{10}O_3$ 202.0630). Anal. Calcd for $C_{12}H_{10}O_3$: C 71.28; H 4.98. Found: C 71.08; H 5.12.

*Preparation of Methyl (E)- and (Z)-((4-hydroxy-6-oxohex-4-enoyl)amino)heptanoate (**2.117**) and the corresponding keto-amide (**2.116**):*



2.116

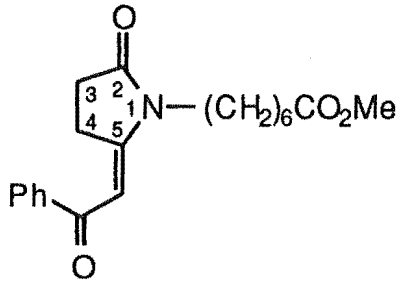


2.117

General method for the preparation of amino acid-derived keto-amides (See page 179) with enollactone (**2.115**) (100mg, 0.50mmol, 1equiv), heptanoic methylester

hydrochloride[E.12] (126mg, 0.64mmol, 1.3equiv) and triethylamine (85µL, 0.64mmol, 1.3equiv) in CH_2Cl_2 (10mL) gave a mixture of the enol-amide (**2.117**) and corresponding keto-amide (**2.116**) in the ratio of 4 : 1, respectively, by ^1H NMR: Yield 166mg, white solid, 93%; FTIR (KBr) 3299, 1737, 1634 and 1568cm^{-1}; ^1H NMR ($CDCl_3$) enol-amide (**1.117**) from mixture δ 1.31 (m, 4H, $NCH_2CH_2C\mathbf{H_2}C\mathbf{H_2}$), 1.49 (m, $NCH_2C\mathbf{H_2}$), 1.60 (m, $C\mathbf{H_2}CH_2CO_2CH_3$), 2.28 (t, J=7.5Hz, $C\mathbf{H_2}CO_2CH_3$), 2.54 (t, J=6.9Hz (**H-2**)$_2$), 2.85 (t, J=6.9Hz, (**H-3**)$_2$), 3.24 (m, $NC\mathbf{H_2}$), 3.66 (s, $C\mathbf{H_3}$), 5.70 (bs, N**H**), 6.21 (s, =C**H**), 7.47 (m, (**Ph**)$_3$), 7.86 (m, (**Ph**)$_2$); ^1H NMR ($CDCl_3$) keto-amide (**1.116**) from mixture δ 4.17 (s, $C\mathbf{H_2}COPh$); HRMS (M) Found 361.1899 (Calcd for $C_{20}H_{27}NO_5$ 361.1889).

(E)-1-Methoxycarbonylhexyl-5-(2-oxo-2-phenylethylidene)-2-pyrrolidinone[E.13] **(2.09)**:



General method A for the preparation of enamino esters (See page 183) with enol-amide (**2.117**) - keto-amide (**2.116**) mixture (60mg, 0.17mmol) and a catalytic quantity of PTSA, in 1, 2-dichloroethane (10mL), and reflux time of 43h: Yield 46mg, oil, 81%; mp 62-63 °C (ether/petroleum ether); ^1H NMR ($CDCl_3$) δ 1.39 (m, 4H, $NCH_2CH_2C\mathbf{H_2}C\mathbf{H_2}$), 1.65 (m, 4H, $NCH_2C\mathbf{H_2}CH_2CH_2C\mathbf{H_2}$), 2.32 (t, J=7.4Hz, $C\mathbf{H_2}CO_2CH_3$), 2.61 (m, (**H-3**)$_2$), 3.44 (m, (**H-4**)$_2$), 3.64 (t, J=7.6Hz, $NC\mathbf{H_2}$), 3.66 (s, $C\mathbf{H_3}$), 6.36 (t, J=1.8Hz, =C**H**), 7.50 (m, (**Ph**)$_3$), 7.90 (m, (**Ph**)$_2$); ^{13}C NMR ($CDCl_3$) δ 24.68, 26.08, 26.34, 26.62, 27.89, 28.67, 33.85, 40.58, 51.48, 96.10, 127.52, 128.50, 131.96, 139.92, 161.96, 174.01, 177.39, 189.80; HRMS (M) Found 343.1779 (Calcd for $C_{20}H_{25}NO_4$ 343.1784).

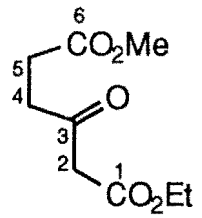
SECTION E.3

CHAPTER 3 EXPERIMENTAL

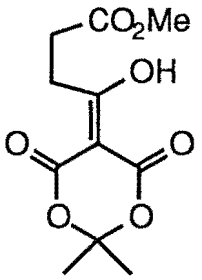
SECTION E.3.1

PREPARATION OF β-KETO ESTER (**3.01**)

Preparation of 1-Ethyl 6-methyl 3-oxohexandioate[E.14] **(3.01)**:



3.01



3.06

To a solution of recently recrystallized Meldrum's acid (2,2-dimethyl-1,3-dioxane-4,6-dione) (1.97g, 0.014mol, 1.03equiv) dissolved in CH_2Cl_2 (5mL) and cooled to 0 °C, dry pyridine (2.69mL, 0.033mol, 2.5equiv) was added over 10min. To this solution, acid chloride[E.15] (**3.05**) (2.00g, 0.013mol, 1equiv) dissolved in CH_2Cl_2 (4mL) was added over 105min and the resulting solution, after stirring at 0 °C for 60min and at 20 °C for 50min, was poured into 2N HCl (8mL) containing crushed ice. The organic layer was removed and the aqueous layer was extracted with CH_2Cl_2 (2x 2mL). The combined CH_2Cl_2 extracts were washed with 2N HCl (2x 2mL), saturated aqueous NaCl solution (3mL), dried ($MgSO_4$) and the solvent was evaporated to give Meldrum's acid compound (**3.06**) as a brown solid (1.95g, 58%), which was used subsequently without further purification: ^{1}H NMR ($CDCl_3$) δ 1.75 (s, $C(C\mathbf{H_3})_2$), 2.75 (t, J=6.8Hz, $C\mathbf{H_2}CO_2CH_3$), 3.45 (t, J=6.8Hz, $C\mathbf{H_2}CH_2CO_2CH_3$), 3.71 (s, $OC\mathbf{H_3}$).

Meldrum's acid compound (**3.06**) (1.95g, 0.0076mol) was dissolved in ethanol (40mL) and refluxed for 2.5h. The solvent was evaporated to yield β-keto ester[E.14] (**3.01**), which was used subsequently without purification, as an oil (1.49g, 95%): ^{1}H NMR ($CDCl_3$) δ 1.29 (t, J=6.9Hz, $CH_2C\mathbf{H_3}$), 2.63 (t, J=6.5Hz, $(\mathbf{H\text{-}5})_2$), 2.88 (t, J=6.5Hz, $(\mathbf{H\text{-}4})_2$), 3.50 (s, $(\mathbf{H\text{-}2})_2$), 3.69 (s, $OC\mathbf{H_3}$), 4.21 (q, J=6.9Hz, $OC\mathbf{H_2}CH_3$); ^{13}C NMR ($CDCl_3$) δ 13.82, 27.44, 37.16, 48.99, 51.68, 61.28, 166.99, 172.83, 201.13.

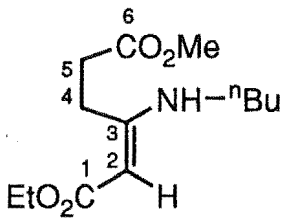
SECTION E.3.2

PREPARATION OF ENAMINES (**3.07-3.10**)

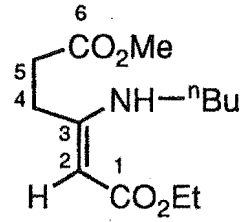
General Method for the Preparation of Protio Enamines (**3.07** and **3.08**):

Glycine ethylester hydrochloride (1.4equiv) and triethylamine (1.4equiv), or butylamine (2equiv) were/was added to β-keto ester (**3.01**) (stated amount, 1equiv), dissolved in benzene, and the mixture was refluxed with azeotropic removal of H_2O for 90min. The solvent was evaporated and the crude enamine (**3.08**, **3.07**, respectively) was purified by radial chromatography using a 4mm silica gel chromatotron plate, eluting with the stated solvent system.

1-Ethyl, 6-methyl (E)- and (Z)-3-(N-butylamino)-2-hexenedioate (**3.07E** and **3.07Z**):



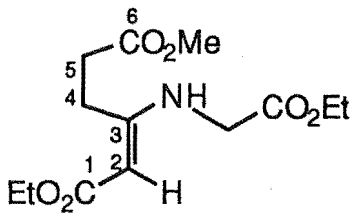
3.07E



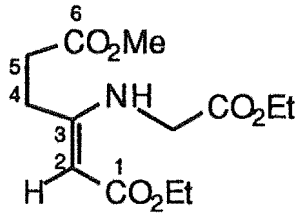
3.07Z

General method with β-keto ester (**3.01**) (100mg, 4.94mmol) and butylamine (1.0mL, 9.87mmol, 2equiv), in benzene (80mL). Elution with 87% petroleum ether/13% ethyl acetate yielded E- and Z-enamines (**3.07E** and **3.07Z**, respectively) in the ratio 22% E : 78% Z, by ^{1}H NMR: 420mg, oil, 47%; FTIR (film) 3389, 3282, 3191, 1740, 1685, 1654 and1608cm^{-1}; ^{1}H NMR ($CDCl_3$) Z isomer (**3.07Z**) from mixture δ 0.96 (t, J=7.3Hz, $CH_2CH_2C\mathbf{H_3}$), 1.27 (t, J=7.1Hz, $OCH_2C\mathbf{H_3}$), 1.40 (m, $NCH_2CH_2C\mathbf{H_2}$), 1.56 (m, $NCH_2C\mathbf{H_2}$), 2.56 (s, 4H, $(\mathbf{H\text{-}4})_2$ and $(\mathbf{H\text{-}5})_2$), 3.23 (q, J=6.5Hz, $NC\mathbf{H_2}$), 3.73 (s, $OC\mathbf{H_3}$), 4.11 (q, J=7.1Hz, $OC\mathbf{H_2}$), 4.44 (s, =C**H**), 8.58 (bs, N**H**); ^{13}C NMR ($CDCl_3$) Z isomer (**3.07Z**) from mixture δ 13.41, 14.26, 19.67, 26.69, 31.66, 32.15, 42.03, 51.46, 57.98, 80.51, 163.23, 170.42, 172.13; HRMS (M) Found 257.1634 (Calcd for $C_{13}H_{23}NO_4$ 257.1637). ^{1}H NMR ($CDCl_3$) indicated the presence of the E isomer (**3.07E**): δ 4.72 (bs, N**H**).

1-Ethyl, 6-methyl (E)- and (Z)-3-(N-ethoxycarbonylmethylamino)-2-hexenedioate (**3.08E** and **3.08Z**):



3.08E



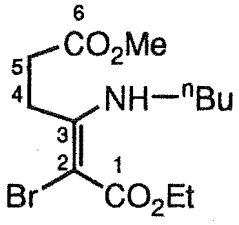
3.08Z

General method with β-keto ester (**3.01**) (500mg, 2.47mmol), glycine ethylester hydrochloride (500mg, 3.58mmol, 1.4equiv) and triethylamine (1.0mL, 9.87mmol, 2equiv), in benzene (40mL). Elution with 80% petroleum ether/20% ethyl acetate yielded E- and Z-enamines (**3.08E** and **3.08Z**, respectively) in the ratio 28% E : 72% Z, by ^{1}H NMR: 319mg, oil, 45%; FTIR (film) 3388, 3291, 1741, 1688, 1657 and 1605cm^{-1}; ^{1}H NMR ($CDCl_3$) Z isomer (**3.08Z**) from mixture δ 1.25 (t, J=7.1Hz, CH_2CH_3), 1.29 (t, J=7.1Hz, CH_2CH_3), 2.52 (m, 4H, (**H-4**)$_2$ and (**H-5**)$_2$), 3.70 (s, OCH_3), 4.01 (d, J=6.1Hz, NCH_2), 4.11 (q, J=7.1Hz, OCH_2), 4.23 (q, J=7.1Hz, OCH_2), 4.55 (s, =C**H**), 8.89 (bt, N**H**); ^{13}C NMR Z isomer (**3.08Z**) from mixture ($CDCl_3$) δ 14.07, 14.45, 26.63, 31.73, 44.34, 51.84, 58.64, 61.50, 83.49, 162.13, 169.66, 170.32, 172.35; HRMS (M) Found 287.1367 (Calcd for $C_{13}H_{21}NO_6$ 287.1369). ^{1}H NMR ($CDCl_3$) indicated the presence of the E isomer (**3.08E**): δ 5.31 (bs, N**H**).

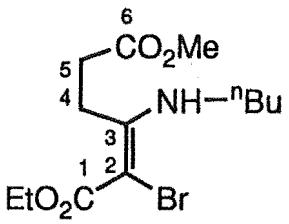
General Method for the Preparation of Bromo Enamines (**3.09** and **3.10**):

NBS (*N*-bromosuccinimide) (1equiv), which had been purified by recrystallization[E.01], was added to enamine (**3.07**, **3.08**) (stated amount, 1equiv) dissolved in THF (20mL), at 0 °C, and the solution was stirred at 0 °C for 15min. The solvent was evaporated and CCl_4 (3mL) was added. The mixture was filtered and the solvent was evaporated to yield enamine (**3.09**, **3.10**, respectively) as an oil, which was used in subsequent steps without further purification.

1-Ethyl, 6-methyl (E)- and (Z)-2-bromo-3-(N-butylamino)-2-hexenedioate (**3.09E** and **3.09Z**):



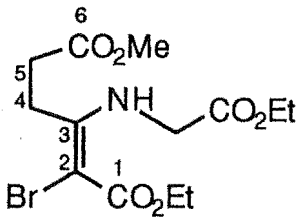
3.09E



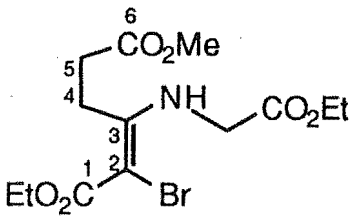
3.09Z

General method with enamine (**3.07**) (95mg, 0.36mmol) and NBS (63mg, 0.35mmol, 1equiv), in THF (20mL), gave E- and Z-enamines (**3.09E** and **3.09Z**, respectively) in the ratio 83% E : 17% Z, by ^{1}H NMR: Yield 119mg, quant; FTIR (film) 3374, 3252, 3146, 1740, 1670, 1634 and 1588cm^{-1}; ^{1}H NMR ($CDCl_3$) E isomer (**3.09E**) from mixture δ 0.94 (t, J=7.3Hz, $CH_2CH_2CH_3$), 1.31 (t, J=7.0Hz, OCH_2CH_3), 1.42 (m, $NCH_2CH_2CH_2$), 1.58 (m, NCH_2CH_2), 2.59 (m, (**H-5**)$_2$), 2.93 (m, (**H-4**)$_2$), 3.28 (m, NCH_2), 3.72 (s, OCH_3), 4.17 (q, J=7.0Hz, OCH_2), 9.28 (bs, NH); ^{13}C NMR ($CDCl_3$) E isomer (**3.09E**) from mixture δ 13.72, 14.46, 19.94, 27.47, 30.82, 32.45, 43.83, 51.98, 60.49, 76.86, 162.39, 167.73, 172.34; HRMS (M) Found 337.0713 (Calcd for $C_{13}H_{22}{}^{81}BrNO_4$ 337.0713), Found 335.0735 (Calcd for $C_{13}H_{22}{}^{79}BrNO_4$ 335.0733). ^{1}H NMR ($CDCl_3$) indicated the presence of the Z isomer (**3.09Z**): δ 5.51 (bs, NH).

1-Ethyl, 6-methyl (E)- and (Z)-2-bromo-3-(N-ethoxycarbonylmethylamino)-2-hexenedioate (**3.10E** and **3.10Z**):



3.10E



3.10Z

General method with enamine (**3.08**) (92mg, 0.32mmol) and NBS (57mg, 0.32mmol, 1equiv), in THF (20mL), gave a mixture of E- and Z-enamines (**3.10E** and **3.10Z**, respectively) in the ratio 75% E : 25% Z, by ^{1}H NMR: Yield 113mg, 96%; FTIR (film) 3357, 3263, 1740, 1641 and 1583cm^{-1}; ^{1}H NMR ($CDCl_3$) E isomer (**3.10E**) from mixture δ 1.30 (t, J=7.1Hz, CH_2CH_3), 1.32 (t, J=7.1Hz, CH_2CH_3), 2.62 (m, (**H-5**)$_2$), 2.83 (m, (**H-4**)$_2$), 3.71 (s, OCH_3), 4.10 (d, J=5.9Hz, NCH_2), 4.20 (q, J=7.1Hz, OCH_2), 4.24 (q, J=7.1Hz, OCH_2), 9.59 (bt, NH); ^{13}C NMR ($CDCl_3$) E isomer (**3.10E**) from mixture δ 14.05, 14.31, 27.53, 30.63, 45.49, 51.91, 60.76, 61.69,

79.47, 161.00, 167.29, 169.39, 172.28; HRMS (M) Found 367.0454 (Calcd for $C_{13}H_{20}{}^{81}BrNO_6$ 367.0455), Found 365.0496 (Calcd for $C_{13}H_{20}{}^{79}BrNO_6$ 365.0474). ^1H NMR ($CDCl_3$) indicated the presence of the Z isomer (**3.10Z**): δ 6.07 (bs, N**H**).

SECTION E.3.3

PREPARATION OF ENAMINO ESTERS (**2.66, 2.71, 3.02-3.03**)

General Method for the Preparation of Enamino Esters (**2.66, 2.71, 3.02-3.03**):

The appropriate enamine (**3.07, 3.09, 3.08, 3.10**) (stated amount, 1equiv), dissolved in THF (3mL), was added to NaH (1equiv) and the solution was stirred at 20 °C for 18h. The solvent was evaporated, CH_2Cl_2 (3mL) was added, the mixture was filtered and the solvent was evaporated to give the enamino ester (**2.66, 2.71, 3.02-3.03**, respectively) as an oil.

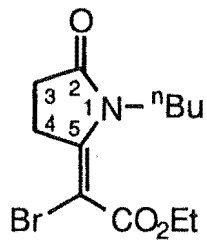
1-Butyl (E)-5-ethoxycarbonylmethylidene-2-pyrrolidinone (**2.66**):

General method with enamine (**3.07**) (50mg, 0.19mmol) and NaH (5mg), in THF (3mL): Yield 37mg, 85%; ^1H NMR ($CDCl_3$) as given earlier (Chapter 2 Experimental). No Z isomer was observed.

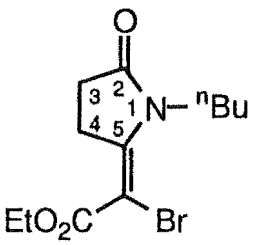
(E)-1-Ethoxycarbonylmethyl-5-ethoxycarbonylmethylidene-2-pyrrolidinone (**2.71**):

General method with enamine (**3.08**) (70mg, 0.24mmol) and NaH (6mg), in THF (3mL): Yield 37mg, 59%; ^1H NMR ($CDCl_3$) as given earlier (Chapter 2 Experimental).

1-Butyl (E)- and (Z)-5-bromoethoxycarbonylmethylidene-2-pyrrolidinone (**3.02**):



3.02E

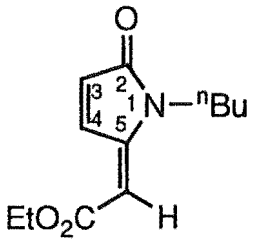


3.02Z

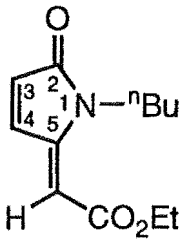
General method with enamine (**3.09**) (33mg, 0.098mmol) and NaH (2mg), in THF (3mL), and a reaction time of 2h gave an oil (16mg) which contained, by ^1H NMR, a mixture of E- and Z-bromo enamino esters (**3.02E** and **3.02Z**, respectively) (80% of mixture; 75% E : 25% Z) and elimination products (**3.11E** and **3.11Z**) (20% of mixture; 75% E : 25% Z). Purification by radial chromatography using a 1mm silica gel chromatotron plate, eluting with 87% petroleum ether/10% ethyl acetate/3% CH_2Cl_2 gave an oil (5mg) which contained, by

^{1}H NMR, a mixture of elimination products (**3.11E** and **3.11Z**) (60% of mixture; 73% E : 27% Z) and E-bromo enamino ester (**3.02E**) (40% of mixture): ^{1}H NMR ($CDCl_3$) E-bromo enamino ester (**3.02E**) from mixture δ 0.89 (t, J=7.2Hz, $CH_2CH_2CH_3$), 1.26 (m, $NCH_2CH_2CH_2$), 1.35 (t, J=7.2Hz, OCH_2CH_3), 1.60 (m, NCH_2CH_2), 2.55 (m, (**H-3**)$_2$), 2.84 (m, (**H-4**)$_2$), 3.77 (t, J=7.6Hz, NCH_2), 4.27 (q, J=7.2Hz, OCH_2); See below for data of elimination products (**3.11E** and **3.11Z**). Further elution gave Z-bromo enamino ester (**3.02Z**) (3mg, 10%): ^{1}H NMR ($CDCl_3$) δ 0.94 (t, J=7.3Hz, $CH_2CH_2CH_3$), 1.33 (m, $NCH_2CH_2CH_2$), 1.34 (t, J=7.1Hz, OCH_2CH_3), 1.57 (m, NCH_2CH_2), 2.53 (m, (**H-3**)$_2$), 3.25 (m, (**H-4**)$_2$), 4.09 (t, J=7.8Hz, NCH_2), 4.25 (q, J=7.1Hz, OCH_2).

Preparation of 1-Butyl (E)- and (Z)-5-ethoxycarbonylmethylidene pyrrolid-3-en-2-one (3.11E and 3.11Z):



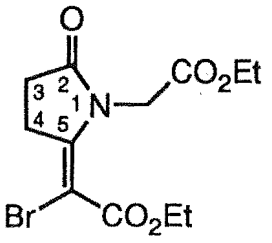
3.11E



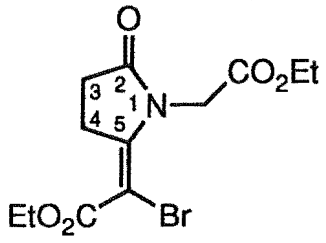
3.11Z

General method with enamine (**3.09**) (51mg, 0.15mmol) and NaH (4mg), in THF (3mL), gave a residue (35mg) which contained, by ^{1}H NMR, elimination products (**3.11E** and **3.11Z**) (70% of mixture; 70% E : 30% Z), and E- and Z-bromo enamino esters (**3.02E** and **3.02Z**, respectively) (30% of mixture; 70% E : 30% Z). This mixture was dissolved in CCl_4 (15mL), containing a catalytic quantity of PTSA, and the solution was heated at 60 °C for 5 days. Purification by radial chromatography using a 1mm silica gel chromatotron plate, eluting with 80% petroleum ether/20% ethyl acetate yielded E and Z isomers of the elimination product (**3.11E** and **3.11Z**, respectively) in the ratio 90% E : 10% Z, by ^{1}H NMR: Yield 14mg, 41%; FTIR (film) 1708, 1635 and 1560cm^{-1}; ^{1}H NMR ($CDCl_3$) E isomer (**3.11E**) from mixture δ 0.94 (t, J=7.3Hz, $CH_2CH_2CH_3$), 1.32 (m, $NCH_2CH_2CH_2$), 1.34 (t, J=7.1Hz, OCH_2CH_3), 1.55 (m, NCH_2CH_2), 3.58 (t, J=7.4Hz, NCH_2), 4.25 (q, J=7.1Hz, OCH_2), 5.54 (d, J=1.7Hz, =CH), 6.30 (dd, J=1.7, 6.0Hz, **H-3**), 8.13 (d, J=6.0Hz, **H-4**); Z isomer (**3.11Z**) from mixture δ 5.35 (s, =CH), 6.27 (d, J=5.8Hz, **H-3**), 6.88 (d, J=5.8Hz, **H-4**); ^{13}C NMR ($CDCl_3$) E isomer (**3.11E**) from mixture δ 13.67, 14.26, 20.02, 30.35, 39.06, 60.59, 99.10, 126.88, 135.45, 151.07, 165.67, 170.29; HRMS (M) Found 223.1210 (Calcd for $C_{12}H_{17}NO_3$ 223.1208).

(E)- and (Z)-5-Bromoethoxycarbonylmethylidene-1-ethoxycarbonylmethyl-2-pyrrolidinone (**3.03E** and **3.03Z**):



3.03E



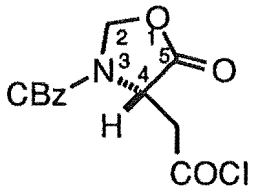
3.03Z

General method with enamine (**3.10**) (50mg, 0.14mmol) and NaH (3mg), in THF (3mL), gave an oil (39mg) which contained, by ^{1}H NMR, a mixture of elimination product (**2.101**) (15% of mixture; 60% E : 40% Z) and E- and Z-bromo enamino esters (**3.03E** and **3.03Z**, respectively) (85% of mixture; 84% Z : 16% E). Some of this mixture (7mg) was purified by preparative tlc on silica, eluting with 80% CH_2Cl_2/15% petroleum ether/5% ethyl acetate to give a fraction (2mg, oil) containing, by ^{1}H NMR, a mixture of elimination products (**2.101E** and **2.101Z**) (70% of mixture; 43% E : 57% Z) and E- bromo enamino ester (**3.03E**) (30% of mixture): ^{1}H NMR ($CDCl_3$) E-bromo enamino ester (**3.03E**) from mixture δ 2.64 (m, (**H-3**)$_2$), 2.97 (m, (**H-4**)$_2$), 4.61 (s, NC**H$_2$**); see page 199 for data of elimination products (**2.101E** and **2.101Z**) (Chapter 2 Experimental). Another fraction (1mg, oil) contained Z-bromo enamino ester (**3.03Z**): ^{1}H NMR ($CDCl_3$) δ 1.30 (t, J=7.1Hz, C**H$_3$**), 1.33 (t, J=7.1Hz, C**H$_3$**), 2.63 (m, (**H-3**)$_2$), 3.36 (m, (**H-4**)$_2$), 4.24 (q, J=7.1Hz, OC**H$_2$**), 4.24 (q, J=7.1Hz, OC**H$_2$**), 4.91 (s, NC**H$_2$**); HRMS (M) Found 335.0190 (Calcd for $C_{12}H_{16}{}^{81}BrNO_5$ 335.0193), Found 333.0177 (Calcd for $C_{12}H_{16}{}^{79}BrNO_5$ 333.0212).

SECTION E.3.4

PREPARATION OF ENAMINO ESTER (**3.04**) WITH THE POTENTIAL FOR PEPTIDE CHAIN EXTENSION IN THE N AND C DIRECTIONS

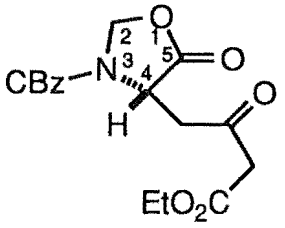
Preparation of (4S) 3-benzyloxycarbonyl-4-chloroformylmethyl-1,3-oxazolidin-5-one (**3.14**):



The benzyloxycarbonyl (CBz) acid[E.16] (**3.13**) (1.89g, 6.77mmol, 1equiv) was dissolved in CH_2Cl_2 (50mL) and the solution was cooled to 0 °C. Freshly distilled oxalyl chloride (8.6mL, 98.6mmol, 15equiv) and a catalytic quantity of DMF were added. The mixture was stirred at 0 °C for 2h and at 20 °C for 16h. The solvent was evaporated, more CH_2Cl_2 (2mL) was added and evaporated (repeated 3 times). Final traces of oxalyl chloride were removed at 1mm to yield acid chloride (**3.14**) as a beige solid (2.02g, 100%) which was used in subsequent steps without further purification: ^{1}H NMR ($CDCl_3$) δ 3.55 (AB_q, J_{AB}=17.2Hz, 1H, C**H**$_a$COCl), 3.86 (bm, 1H, C**H**$_b$COCl), 4.33 (m, **H-4**), 5.17 (AB_q, J_{AB}=12.7Hz, 1H, C**H**$_a$Ph), 5.23 (AB_q, J_{AB}=12.7Hz, 1H, C**H**$_b$Ph), 5.34 (m, 1H, (**H-2**)$_a$), 5.50 (bs, 1H, (**H-2**)$_b$), 7.37 (m, (**Ph**)$_5$).

ENAMINE ROUTE TO ENAMINO ESTER (**3.04**)

Preparation of (4S) 3-Benzyloxycarbonyl-4-(3-ethoxycarbonyl-2-oxopropyl)-1,3-oxazolidin-5-one (**3.15**):

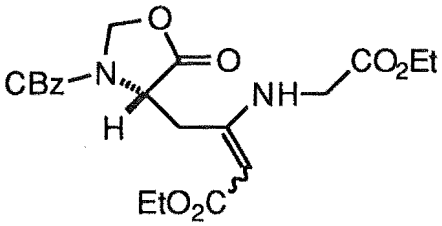


Pyridine (53μL, 0.65mmol, 2equiv) and Meldrum's acid (47mg, 0.33mmol, 1equiv) were added to acid chloride (**3.14**) (107mg, 0.36mmol, 1.1equiv), dissolved in CH_2Cl_2 (5mL), at 0

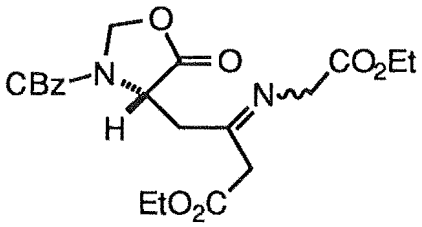
°C. The solution was stirred at 0 °C for 1h, then at 20 °C for 1h. The solvent was evaporated to give an oil (207mg), used subsequently without further purification, containing acylated Meldrum's acid: ^{1}H NMR ($CDCl_3$) δ 1.72 (s, $C(CH_3)_2$), 3.04 (m, 1H, $C4CH_a$), 3.36 (bm, 1H, $C4CH_b$), 4.34 (bs, **H-4**), 5.18 (m, CH_2Ph), 5.32 (bs, 1H, $(H\text{-}2)_a$), 5.53 (bs, 1H, $(H\text{-}2)_b$), 7.36 (m, $(Ph)_5$).

Acylated Meldrum's acid (assumed 0.33mmol) was dissolved in ethanol (10mL) and refluxed for 2h. The solvent was evaporated. Purification by radial chromatography using a 1mm silica gel chromatotron plate, eluting with 80% CH_2Cl_2/20% ethyl acetate gave oxazolidinone (**3.15**) as an oil (86mg, 75%), which was used in subsequent steps without further purification: ^{1}H NMR ($CDCl_3$) δ 1.26 (t, J=7.2Hz, CH_3), 3.21 (m, 1H, $C4CH_a$), 3.44 (s, CH_2CO_2Et), 3.65 (bm, 1H, $C4CH_b$), 4.18 (q, J=7.2Hz, OCH_2), 4.30 (s, **H-4**), 5.19 (s, CH_2Ph), 5.38 (d, J=3.0Hz, 1H, $(H\text{-}2)_a$), 5.51 (s, 1H, $(H\text{-}2)_b$), 7.36 (m, $(Ph)_5$); ^{13}C NMR ($CDCl_3$) δ 13.87, 42.24, 48.54, 50.60, 61.53, 67.77, 78.21, 128.14, 128.48, 128.57, 135.22, 152.46, 166.18, 171.66, 199.95; HRMS (M) Found 349.1165 (Calcd for $C_{17}H_{19}NO_7$ 349.1161).

*Preparation of enamine (**3.16**)/imine (**3.21**):*



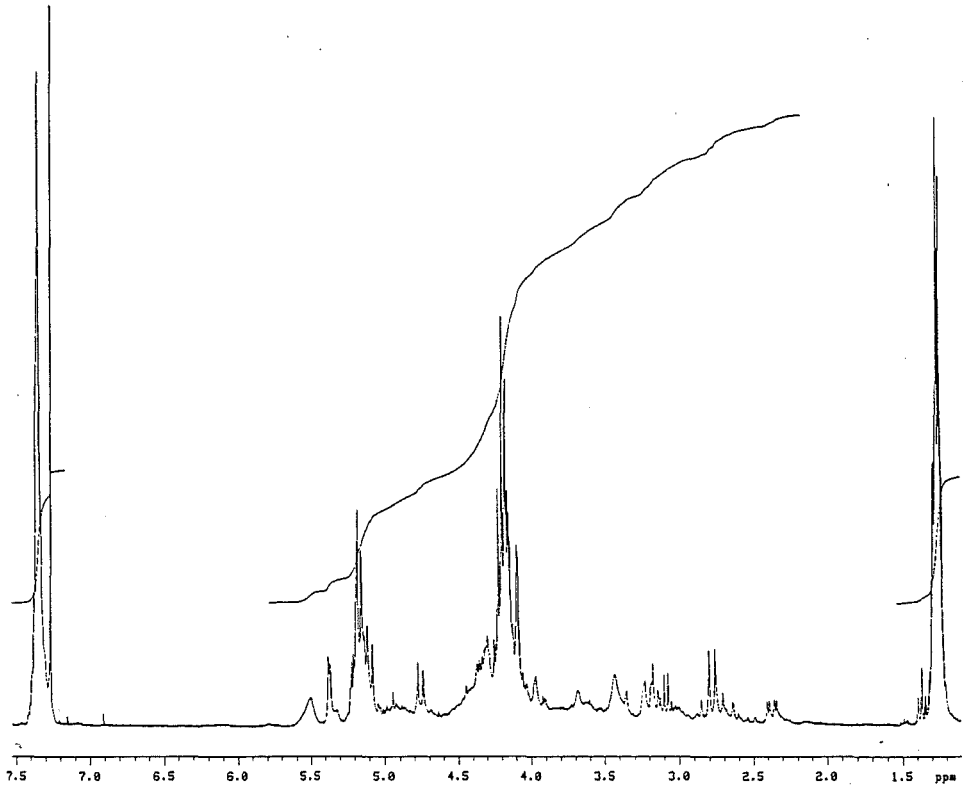
3.16



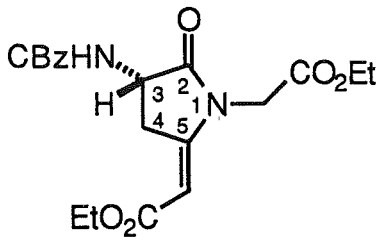
3.21

Glycine ethylester hydrochloride (65mg, 0.47mmol, 1.5equiv) and triethylamine (62μL, 0.47mmol, 1.5equiv) were added to β-keto ester (**3.15**) (109mg, 0.31mmol, 1equiv), dissolved in benzene (24mL), and the mixture was refluxed, with azeotropic removal of H_2O, for 90min. The mixture was filtered and the solvent was evaporated to yield an oil (116mg, 86%) which was used in subsequent steps with further purification: ^{1}H NMR ($CDCl_3$) shown below (Spectrum E.01). HRMS (M) Found 434.1689 (Calcd for $C_{21}H_{26}N_2O_8$ 434.1694).

Spectrum E.01

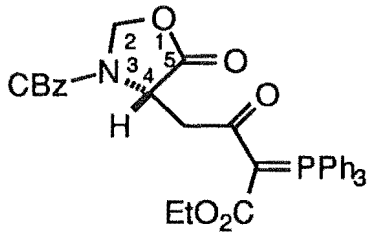


Preparation of (3S) (E)-3-Benzyloxycarbonylamino-1-ethoxycarbonylmethyl-5-ethoxycarbonylmethylidine-2-pyrrolidinone (**3.04**):



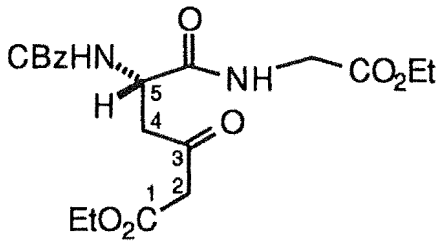
Enamine (**3.16**)/imine (**3.21**) (51mg, 0.12mmol) was heated at 150 °C, at 1mm, for 1h. Purification by radial chromatography using a 1mm silica gel chromatotron plate, eluting with 80% CH_2Cl_2/20% ethyl acetate gave enamino ester (**3.04**) as an oil (31mg, 65%): FTIR (film) 3350, 1801, 1714, 1632 and 1530cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, J=7.1Hz, C**H**$_3$), 1.29 (t, J=7.1Hz, C**H**$_3$), 3.11 (dd, J=6.9, 19.0Hz, 1H, (**H-3**)$_a$), 3.91 (dd, J=9.7, 19.0Hz, 1H, (**H-3**)$_b$), 4.12-4.26 (m, 6H, 2x C**H**$_2$CH$_3$ and NC**H**$_2$), 4.43 (m, **H-4**), 5.12 (m, 3H, C**H**$_2$Ph and =C**H**), 5.39 (bs, N**H**), 7.35 (m, (**Ph**)$_5$); ^{13}C NMR ($CDCl_3$) δ 14.07, 14.36, 32.85, 42.11, 50.06, 59.92, 62.12, 67.39, 93.50, 128.19, 128.33, 128.58, 128.64, 154.84, 155.78, 166.27, 166.49, 173.74; HRMS (M) Found 404.1577 (Calcd for $C_{20}H_{24}N_2O_7$ 404.1583); $(\alpha)_D^{20}$ = -13° (c 0.35; CH_2Cl_2).

Preparation of (4S) 3-benzyloxycarbonyl-4-(3-ethoxycarbonyl-2-oxo-3-triphenylphosphoranylidenylpropyl)-1,3-oxazolidin-5-one[E.17] (**3.17**):



Acid chloride (**3.14**) (107mg, 0.36mmol, 1equiv) was dissolved in CH_2Cl_2 (50mL) and cooled to 0 °C. $Ph_3P{=}CHCO_2Et$[E.03] (251mg, 0.72mmol, 2equiv) was added and the solution was stirred at 0 °C for 30min and at 20 °C for 30min. The solvent was evaporated and the residue was purified by radial chromatography using a 2mm silica gel chromatotron plate, eluting with 55% ethyl acetate/45% petroleum ether to give phosphorane (**3.17**) as a colourless solid (187mg, 84%), which was used subsequently without further purification: ^{1}H NMR ($CDCl_3$) δ 0.73 (bt, C**H**$_3$), 3.39 (AB$_q$, J_{AB}=17.5Hz, 1H, C4C**H**$_a$), 3.77 (q, J=7.1Hz, OC**H**$_2$CH$_3$), 4.20-4.32 (m, 3H, C4C**H**$_b$, **H-4** and (**H-2**)$_a$), 5.16 (m, C**H**$_2$Ph), 5.31 (AB$_q$, J_{AB}=12.0Hz, 1H, (**H-2**)$_b$), 7.49 (m, (**Ph**)$_{20}$).

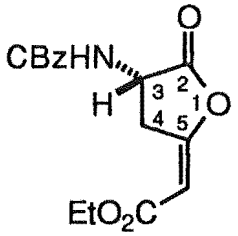
Preparation of 1-Ethyl (5S) 5-benzyloxycarbonylamino-3-oxo-2-triphenylphosphoranylidenehexandioate[E.17] (**3.18**):



1N NaOH (1.5mL, 1.5mmol, 6equiv) was added to a stirred solution of phosphorane (**3.17**) (150mg, 0.25mmol), in methanol (3mL), at 20 °C. After 4h the solution was acidified to pH 3 with 1N HCl, the solvent was evaporated and the residue was extracted with ethyl acetate (2x 5mL). The combined ethyl acetate extracts were dried ($MgSO_4$) and the solvent was evaporated to yield acid (**3.18**) as a colourless solid (145mg, quant): ^{1}H NMR ($CDCl_3$) δ

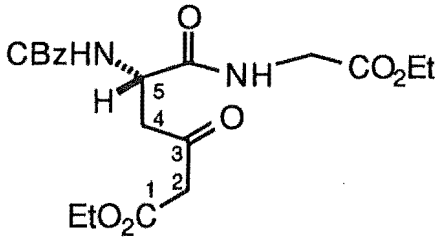
0.67 (t, J=7.1Hz, CH_3), 3.13 (m, 1H, **(H-4)**$_a$), 3.73 (m, OCH_2CH_3), 4.02 (m, 1H, **(H-4)**$_b$), 4.55 (m, **H-5**), 5.11 (m, OCH_2Ph), 5.94 (d, J=6.5Hz, **NH**), 7.29-7.69 (m, **(Ph)**$_{20}$).

Preparation of (4S) Ethyl (E)-3-benzyloxycarbonylamino-5-ethoxycarbonylmethylidene-2-tetrahydrofuranone[E.17] **(3.19)**:



Acid (**3.18**) (30mg, 0.05mmol) was dissolved in $CHCl_3$ (5mL) and refluxed for 48h. The solvent was evaporated and the residue was purified by radial chromatography using a 1mm silica gel chromatotron plate, eluting with 85% CH_2Cl_2/15% ethyl acetate to give E-enollactone (**3.19**) as a colourless oil (12mg, 78%): ^{1}H NMR ($CDCl_3$) δ 1.28 (t, J=7.2Hz, CH_3), 3.25 (dd, J=7.4, 18.5Hz, 1H, **(H-4)**$_a$), 3.89 (dd, J=10.5, 18.5Hz, 1H, **(H-4)**$_b$), 4.18 (q, J=7.2Hz, OCH_2CH_3), 4.36 (bq, J=9.4Hz, **H-3**), 5.13 (m, OCH_2Ph), 5.56 (bs, **NH**), 5.74 (s, =**CH**), 7.35 (m, **(Ph)**$_5$).

Preparation of Ethyl (5S) 5-benzyloxycarbonylamino-5-(N-(1-ethoxycarbonylmethyl)carbamoyl)-3-oxopentanoate **(3.20)**:



Glycine ethylester hydrochloride (9mg, 0.068mmol, 1.2equiv) and triethylamine (9μL, 0.068mmol, 1.2equiv) were added to enollactone (**3.19**) (18mg, 0.056mmol, 1equiv), dissolved in CH_2Cl_2 (5mL), and the mixture was stirred at 20 °C for 16h. The solution was washed with H_2O (5mL), dried ($MgSO_4$) and the solvent evaporated to yield keto-amide (**3.20**) as a pale yellow oil (15mg, 63%) which was used in subsequent steps without further purification: FTIR (film) 3344, 1720 and 1530cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.26 (t, J=7.1Hz, CH_3), 1.27 (t, J=7.1Hz, CH_3), 2.95 (dd, J=6.0, 18.0Hz, 1H, **(H-4)**$_a$), 3.29 (dd, J=4.0, 18.0Hz, 1H, **(H-4)**$_b$), 3.50 (s,

(**H-2**)$_2$), 3.98 (dd, J=1.8, 5.4Hz, NHC**H$_2$**), 4.18 (q, J=7.1Hz, C**H$_2$**CH$_3$), 4.20 (q, J=7.1Hz, C**H$_2$**CH$_3$), 4.66 (m, **H-5**), 5.13 (s, C**H$_2$**Ph), 5.94 (d, J=8.4Hz, CBzN**H**), 6.97 (bs, N**H**CH$_2$), 7.36 (m, (**Ph**)$_5$); ^{13}C NMR ($CDCl_3$) δ 14.03, 14.09, 41.50, 44.00, 49.37, 50.86, 61.53, 61.58, 67.37, 128.14, 128.30, 128.57, 135.90, 156.13, 166.70, 169.29, 170.66, 202.26; HRMS (M) Found 422.1693 (Calcd for $C_{20}H_{26}N_2O_8$ 422.1689).

*Preparation of (3S) (E)-3-Benyloxycarbonylamino-1-ethoxycarbonylmethyl-5-ethoxycarbonylmethylidene-2-pyrrolidinone (**3.04**):*

Keto-amide (**3.20**) (15mg, 0.036mmol) and PTSA (5mg) dissolved in 1, 2-dichloroethane (6mL) were refluxed, with azeotropic removal of H_2O, for 4h. The solution was cooled to 20 °C, washed with H_2O (2mL), dried ($MgSO_4$) and the solvent evaporated to yield enamino ester (**3.16**) as a yellow oil (11mg, 78%): ^{1}H NMR ($CDCl_3$) as given above.

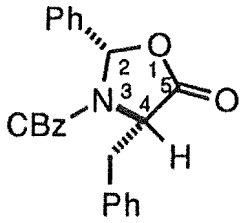
SECTION E.4

CHAPTER 4 EXPERIMENTAL

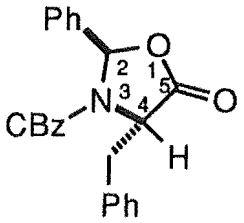
SECTION E.4.1

PREPARATION OF CBz OXAZOLIDINONES (**4.11, 4.14-4.15, 4.18-4.19, 4.21**)

Preparation of (2R,4S)-4-Benzyl-3-benzyloxycarbonyl-2-phenyl-1,3-oxazolidin-5-one[E.18] (**4.11**):



4.11



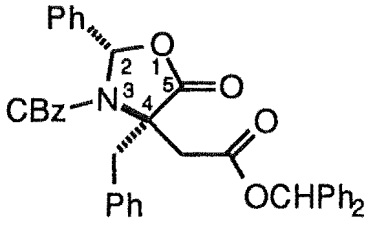
4.12

METHOD A[E.19]: The Schiff base salt[E.19-E.20] (**4.09**) of (S)-phenylalanine (0.121mol), in CH_2Cl_2 (500mL), was cooled to -20 °C and benzyl chloroformate (17.0mL, 0.121mol) was added. The mixture was stirred at -20 °C for 12h and then at 4 °C for 3 days. The solvent was evaporated and the residue partitioned between ethyl acetate (500mL) and aqueous 5% $NaHCO_3$ solution (500mL). The organic layer was extracted, washed with aqueous 5% $KHSO_4$ solution (500mL) and H_2O (500mL), dried (Na_2SO_4) and the solvent was evaporated to yield an oil which contained, by ^1H NMR, 95% cis oxazolidinone (**4.11**) and 5% trans oxazolidinone (**4.12**). Purification by silica column chromatography, eluting with 80% petroleum ether/20% ethyl acetate yielded cis oxazolidinone (**4.11**) as a white solid (22.06g, 47%): mp 124-128 °C (ethyl acetate/petroleum ether) (Lit[E.18] mp 109-112 °C); ^1H NMR ($CDCl_3$) δ 3.19-3.43 (bm, C4C**H**$_2$Ph), 4.66 (dd, J=4.0, 5.9Hz, **H-4**), 5.05 (AB_q, J_{AB}=12.1Hz, 1H, OC**H**$_a$Ph), 5.16 (AB_q, J_{AB}=12.1Hz, 1H, OC**H**$_b$Ph), 6.45 (bs, **H-2**), 7.06-7.33 (m, (**Ph**)$_{15}$); ^{13}C NMR ($CDCl_3$) δ 36.38, 58.13, 67.76, 89.10, 126.55, 127.10, 127.98, 128.11, 128.27, 128.39, 128.58, 129.16, 130.14, 135.13, 136.12, 153.74, 170.90; $(\alpha)_D^{20}$ = +40° (c 1.0; CH_2Cl_2). Selected ^1H NMR data for the trans oxazolidinone (**4.12**); ($CDCl_3$) δ 3.11 (m, C**H**$_2$Ph), 4.71 (m, **H-4**).

METHOD B[E.18]: (S)-CBz phenylalanine (**4.10**) (10.0g, 0.033mol, 1equiv), benzaldehyde (6.8mL, 0.067mol, 2equiv) and PTSA (6.36g, 0.033mol, 1equiv) dissolved in 1,1,1-

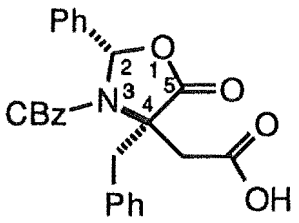
trichloroethane (135mL) were refluxed, with azeotropic removal of H_2O, for 18h to give crude cis and trans oxazolidinones (**4.11** and **4.12**, respectively) in the ratio of 1 : 1, by ^1H NMR. Cis oxazolidinone (**4.11**) was purified as above: Yield 21%; mp and ^1H NMR ($CDCl_3$) as given above.

*Preparation of (2R,4S) 4-Benzyl-3-benzyloxycarbonyl-4-(diphenylmethoxycarbonylmethyl)-2-phenyl-1,3-oxazolidin-5-one (**4.14**):*



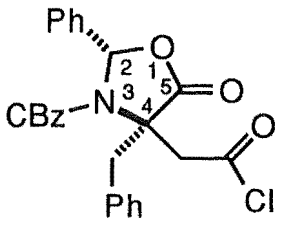
Oxazolidinone (**4.11**) (7.85g, 0.020mol 1equiv) was dissolved in THF (200mL) and the solution was cooled to -78 °C. Lithium hexamethyldisilazide (LiHMDS) (22.3mL of 1M solution in THF, 0.022mol, 1.1equiv) was added and the solution was stirred at -78 °C for 7min. $BrCH_2CO_2CHPh_2$[E.21] (6.43g, 0.0211mol, 1.04equiv) was added and the resulting yellow solution was stirred at -78 °C for 2h and was then allowed to warm to 20 °C over 16h. The THF was evaporated and the residue partitioned between saturated aqueous NH_4Cl solution (100mL) and ether (100mL). The aqueous layer was separated and extracted with ether (2x 100mL). The combined ether extracts were dried (Na_2SO_4) and evaporated to give the crude oxazolidinone (**4.14**) as a yellow oil (12.3g, quant) which was used in subsequent steps without further purification: ^1H NMR ($CDCl_3$) δ 3.19 (AB_q, J_{AB}=17.4Hz, 1H, C**H**$_a$$CO_2CHPh_2$), 3.25 ($AB_q$, J_{AB}=13.2Hz, 1H, C4C**H**$_a$Ph), 3.56 (AB_q, J_{AB}=13.2Hz, 1H, C4C**H**$_b$Ph), 3.89 (AB_q, J_{AB}=17.4Hz, 1H, C**H**$_b$$CO_2CHPh_2$), 4.66 ($AB_q$, J_{AB}=12.7Hz, 1H, OC**H**$_a$Ph), 5.02 (AB_q, J_{AB}=12.7Hz, 1H, OC**H**$_b$Ph), 5.95 (s, **H-2**), 5.99 (d, J=7.3Hz, (**Ph**)$_2$), 6.61 (d, J=7.3Hz, (**Ph**)$_2$), 6.91 (s, C**H**Ph_2), 6.93 (m, (**Ph**)$_2$), 7.07-7.36 (m, (**Ph**)$_{19}$). ^1H NMR indicated the presence of < 5% of the 2R,4R oxazolidinone (**4.16**).

*Preparation of (2R,4S) 4-Benzyl-3-benzyloxycarbonyl-4-carboxymethyl-2-phenyl-1,3-oxazolidin-5-one (**4.18**):*



The benzhydryl oxazolidinone (**4.14**) (0.020mol, 1equiv) was dissolved in CH_2Cl_2 (500mL) and the solution was cooled to 0 °C. TFA (trifluoroacetic acid) (31mL, 0.406mol, 20equiv) was added and the solution was stirred at 0 °C for 2h, then was diluted to 1L with CH_2Cl_2 and washed with H_2O (3x 1L). (Attempted purification by extraction into aqueous 5% $NaHCO_3$ solution produced an emulsion.) The organic layer was dried ($MgSO_4$) and the solvent evaporated to yield acid (**4.18**) as a yellow oil which was crystallized from ethyl acetate/petroleum ether (2.85g, white crystals, 32%): mp 181-185 °C; FTIR (KBr) 3415, 1794, 1738 and 1674cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.13 (AB_q, J_{AB}=18.1Hz, 1H, C**H**$_a$$CO_2H$), 3.25 ($AB_q$, J_{AB}=13.5Hz, 1H, C4C**H**$_a$Ph), 3.57 (AB_q, J_{AB}=13.5Hz, 1H, C4C**H**$_b$Ph), 3.87 (AB_q, J_{AB}=18.1Hz, 1H, C**H**$_b$$CO_2H$), 4.82 ($AB_q$, J_{AB}=12.2Hz, 1H, OC**H**$_a$Ph), 5.11 (AB_q, J_{AB}=12.2Hz, 1H, OC**H**$_b$Ph), 6.13 (d, J=7.3Hz, (**Ph**)$_2$), 6.30 (s, **H-2**), 6.68 (d, J=7.4Hz, (**Ph**)$_2$), 6.96-7.41 (m, (**Ph**)$_{11}$); ^{13}C NMR ($CDCl_3$) δ 38.85, 41.85, 65.00, 67.45, 90.56, 127.71, 127.96, 128.21. 129.13, 129.35, 130.82, 134.65, 135.16, 135.40, 152.24, 172.72, 174.75; HRMS (M) Found 445.1539 (Calcd for $C_{26}H_{23}NO_6$ 445.1525). Anal. Calcd for $C_{26}H_{23}NO_6$: C 70.10; H 5.20; N 3.14. Found: C 69.36; H 5.42; N 3.12.

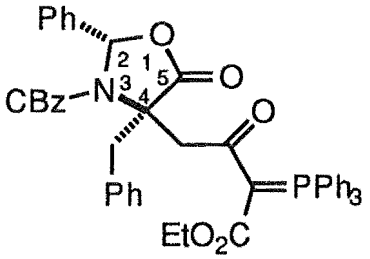
*Preparation of (2R,4S) 4-Benzyl-3-benzyloxycarbonyl-4-chloroformylmethyl-2-phenyl-1,3-oxazolidin-5-one (**4.19**):*



The acid (**4.18**) (402mg, 0.90mmol, 1equiv) was dissolved in CH_2Cl_2 (32mL) and the solution was cooled to 0 °C. Freshly distilled oxalyl chloride (0.39mL, 4.51mmol, 5equiv) and a catalytic quantity of DMF were added. The mixture was stirred at 0 °C for 2h and at 20 °C for 16h. The solvent was evaporated, more CH_2Cl_2 (2mL) was added and evaporated

(repeated 3 times). Final traces of oxalyl chloride were removed at 1mm to yield acid chloride (**4.19**) as a beige solid (418mg, 100%), which was used in subsequent steps without further purification: ^{1}H NMR ($CDCl_3$) δ 3.20 (AB_q, J_{AB}=13.2Hz, 1H, C4C**H**$_a$Ph), 3.51 (AB_q, J_{AB}=13.2Hz, 1H, C4C**H**$_b$Ph), 3.61 (AB_q, J_{AB}=19.1Hz, 1H, C**H**$_a$COCl), 4.37 (AB_q, J_{AB}=19.1Hz, 1H, C**H**$_b$COCl), 4.84 (AB_q, J_{AB}=12.2Hz, 1H, OC**H**$_a$Ph), 5.10 (AB_q, J_{AB}=12.2Hz, 1H, OC**H**$_b$Ph), 6.12 (d, J=7.3Hz, (**Ph**)$_2$), 6.30 (s, **H-2**), 6.72 (d, J=7.8Hz, (**Ph**)$_2$), 7.01 (m, (**Ph**)$_4$), 7.23 (m, (**Ph**)$_5$), 7.37 (m, (**Ph**)$_2$).

*Preparation of (2R,4S) 4-Benzyl-3-benzyloxycarbonyl-4-(3-ethoxycarbonyl-2-oxo-3-triphenylphosphoranylidenylpropyl)-2-phenyl-1,3-oxazolidin-5-one (**4.15**):*



METHOD A: Acid chloride (**4.19**) (412mg, 0.89mmol, 1equiv) was dissolved in CH_2Cl_2 (32mL) and cooled to 0 °C. $Ph_3P{=}CHCO_2Et$[E.03] (619mg, 1.78mmol, 2equiv) was added and the solution was stirred at 0 °C for 1.5h and at 20 °C for 4.5h. The solvent was evaporated and the residue was purified by radial chromatography using a 4mm silica gel chromatotron plate, eluting with 55% petroleum ether/45% ethyl acetate to give oxazolidinone (**4.15**) as a 1 : 1 mixture of 2 diastereoisomers (colourless solid, quant): mp 209-211 °C (ethyl acetate/petroleum ether, 691mg, colourless crystals); FTIR (KBr) 1790, 1710, 1666 and 1559cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.70 (t, J=7.1Hz, C**H**$_3$), 0.77 (t, J=7.1Hz, C**H**$_3$), 3.27 (AB_q, J_{AB}=13.4Hz, 1H, C4C**H**$_a$Ph), 3.29 (AB_q, J_{AB}=13.2Hz, 1H, C4C**H**$_a$Ph), 3.46 (AB_q, J_{AB}=13.2Hz ,1H, C4C**H**$_b$Ph), 3.51 (AB_q, J_{AB}=18.0Hz, 1H, C4C**H**$_a$CO), 3.59 (AB_q, J_{AB}=18.6Hz, 1H, C4C**H**$_a$CO), 3.67 (AB_q, J_{AB}=13.4Hz, 1H, C4C**H**$_b$Ph), 3.78 (m, 4H, 2x OC**H**$_2$CH$_3$), 4.69 (AB_q, J_{AB}=18.6Hz, 1H, C4C**H**$_b$CO), 4.74 (AB_q, J_{AB}=12.2Hz, 1H, OC**H**$_a$Ph), 4.76 (AB_q, J_{AB}=18.0Hz, 1H, C4C**H**$_b$CO), 5.09 (AB_q, J_{AB}=12.2Hz, 1H, OC**H**$_b$Ph), 5.21 (AB_q, J_{AB}=12.2Hz, 1H, OC**H**$_a$Ph), 5.29 (s, **H-2**), 5.29 (AB_q, J_{AB}=12.2Hz, 1H, OC**H**$_b$Ph), 5.33 (s, **H-2**), 5.86 (d, J=7.4Hz, (**Ph**)$_2$), 6.01 (d, J=7.3Hz, (**Ph**)$_2$), 6.57 (d, J=7.4Hz, (**Ph**)$_2$), 6.92 (q, J=7.6Hz, (**Ph**)$_4$), 7.10-7.67 (m, (**Ph**)$_{40}$); ^{31}P NMR ($CDCl_3$) δ 18.1; ^{13}C NMR ($CDCl_3$) δ 13.75, 41.93, 42.74, 45.95 (d, J=7.6Hz), 48.01 (d, J=7.1Hz), 58.28, 58.54, 65.22, 65.72,

66.63, 67.24, 71.08 (d, J=109.3Hz), 71.24 (d, J=110.8Hz), 89.35, 89.50, 125.56 (d, J=93.7Hz), 125.89 (d, J=93.1Hz), 126.88, 127.01, 127.33, 127.51, 127.62, 127.79, 127.91, 127.97, 128.25, 128.45 (d, J=12.0Hz), 128.60, 128.62 (d, J=12.6Hz), 128.64, 128.70, 129.08, 130.80, 131.63 (d, J=2.5Hz), 133.15 (d, J=10.1Hz), 133.20 (d, J=9.6Hz), 135.40, 135.65, 135.71, 135.98, 136.02, 136.46, 151.69, 152.23, 167.33 (d, J=14.1Hz), 167.45 (d, J=14.1Hz), 173.96, 174.13, 192.14 (d, J=6.0Hz), 192.22 (d, J=5.1Hz); HRMS (FAB, M+1) Found 776.2776 (Calcd for $C_{48}H_{43}NO_7P$ 776.2777); $(\alpha)_D^{20}$ = -4 (c 1.5; CH_2Cl_2). Anal. Calcd for $C_{48}H_{42}NO_7P$: C 74.31; H 5.46; N 1.81. Found: C 74.08; H 5.36; N 1.79.

METHOD B: Oxazolidinone (**4.11**) (100mg, 0.26mmol, 1equiv) was dissolved in THF (10mL) and cooled to -78 °C. LiHMDS (0.28mL, 0.28mmol, 1.1equiv) was added and the resulting yellow solution was stirred at -78 °C for 7min. $BrCH_2COC(PPh_3)CO_2Et$ (127mg, 0.27mmol, 1.05equiv) was added and the solution was stirred at -78 °C for 2h and was then allowed to warm to 20 °C over 16h. The THF was evaporated and the residue partitioned between saturated aqueous NH_4Cl solution (10mL) and CH_2Cl_2 (10mL). The aqueous layer was separated and extracted with CH_2Cl_2 (2x 10mL). The combined CH_2Cl_2 extracts were dried ($MgSO_4$) and evaporated. Further purification by radial chromatography using a 2mm silica gel chromatotron plate, eluting with 55% petroleum ether/45% ethyl acetate yielded oxazolidinone (**4.15**) as a white solid (52mg, 26%): ^1H NMR ($CDCl_3$) as given above.

Preparation of $BrCH_2COC(PPh_3)CO_2Et$:

A procedure similar to that reported was used[E,22]. Freshly distilled bromo acetyl bromide (100μL, 1.15mmol, 1equiv) was added to $Ph_3P{=}CHCO_2Et$ (800mg, 2.30mmol, 2equiv) dissolved in CH_2Cl_2 (12mL) and cooled to 0 °C. The solution was stirred at 0 °C for 1h, then 20 °C for 4.5h. The solvent was evaporated and the residue was purified by radial chromatography using a 4mm silica gel chromatotron plate eluting with 95% CH_2Cl_2/5% ethyl acetate to yield $BrCH_2COC(PPh_3)CO_2Et$ as a pale pink solid (108mg, 20%). $BrCH_2COC(PPh_3)CO_2Et$ was purified further by recrystallization from ethyl

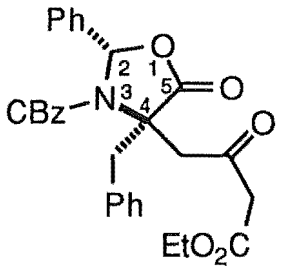
acetate/petroleum ether: ^{1}H NMR ($CDCl_3$) δ 0.67 (t, J=7.1Hz, $\mathbf{CH_3}$), 3.75 (q, J=7.1Hz, $OC\mathbf{H_2}$), 4.56 (s, $C\mathbf{H_2}Br$), 7.43-7.57 (m, $(\mathbf{Ph})_9$), 7.64-7.71 (m, $(\mathbf{Ph})_6$).

High Temperature ^{1}H NMR for (2R,4S) 4-Benzyl-3-benzyloxycarbonyl-4-(3-ethoxycarbonyl-2-oxo-3-triphenylphosphoranylidenylpropyl)-2-phenyl-1,3-oxazolidin-5-one (**4.15**):

^{1}H NMR (d_6-DMSO, 23 °C) δ 0.65 (t, J=6.8Hz, $\mathbf{CH_3}$), 0.67 (t, J=6.8Hz, $\mathbf{CH_3}$), 3.19-3.58 (m, 6H, 2xC4C$\mathbf{H_2}$Ph and 2x C4C$\mathbf{H_a}$CO), 3.73 (m, 4H, 2x $OC\mathbf{H_2}CH_3$), 4.78 (AB_q, J_{AB}=17.6Hz, 1H, C4C$\mathbf{H_b}$CO), 4.81 (AB_q, J_{AB}=18.0Hz, 1H, C4C$\mathbf{H_b}$CO), 4.96 (AB_q, J_{AB}=12.7Hz, 1H, $OC\mathbf{H_a}$Ph), 5.20 (AB_q, J_{AB}=12.7Hz, 1H, $OC\mathbf{H_b}$Ph), 5.20 (s, **H-2**), 5.22 (s, **H-2**), 5.30 (AB_q, J_{AB}=12.2Hz, 1H, $OC\mathbf{H_a}$Ph), 5.34 (AB_q, J_{AB}=12.2Hz, 1H, $OC\mathbf{H_b}$Ph), 5.93 (d, J=7.3Hz, $(\mathbf{Ph})_2$), 6.00 (d, J=7.8Hz, $(\mathbf{Ph})_2$), 6.66 (d, J=6.8Hz, $(\mathbf{Ph})_2$), 7.03-7.76 (m, $(\mathbf{Ph})_{44}$).

^{1}H NMR (d_6-DMSO, 85 °C) δ 0.77 (t, J=7.3Hz, $\mathbf{CH_3}$), 3.26 (AB_q, J_{AB}=13.2Hz, 1H, C4C$\mathbf{H_a}$Ph), 3.37 (AB_q, J_{AB}=17.6Hz, 1H, C4C$\mathbf{H_a}$CO), 3.53 (AB_q, J_{AB}=13.2Hz, 1H, C4C$\mathbf{H_b}$Ph), 3.77 (m, $OC\mathbf{H_2}CH_3$), 4.74 (AB_q, J_{AB}=17.6Hz, 1H, C4C$\mathbf{H_b}$CO), 5.18 (m, $OC\mathbf{H_2}$Ph), 5.43 (s, **H-2**), 6.16 (d, J=7.3Hz, $(\mathbf{Ph})_2$), 7.10 (m, $(\mathbf{Ph})_4$), 7.37 (m, $(\mathbf{Ph})_6$), 7.68 (m, $(\mathbf{Ph})_{13}$).

Preparation[E.23] *of (2R,4S) 4-Benzyl-3-benzyloxycarbonyl-4-(3-ethoxycarbonyl-2-oxopropyl)-2-phenyl-1,3-oxozolidin-5-one* (**4.21**):



Carbonyl diimidazole (CDI) (175mg, 1.08mmol, 1.2equiv) was added to acid (**4.18**) (400mg, 0.90mmol, 1equiv) in THF (40mL). After stirring at 20 °C for 2h, freshly prepared magnesium diethyl malonate (257mg, 0.90mmol, 1equiv) was added and the mixture was stirred at 20 °C for 19h. The mixture was concentrated to 5mL, diluted with ethyl acetate (35mL) and washed with H_2O (40mL), 5% aqueous $KHSO_4$ solution (40mL), 5% aqueous $NaHCO_3$ solution (40mL) and 10% aqueous NaCl solution (40mL). The organic layer was dried (Na_2SO_4) and the solvent evaporated. Purification by radial chromatography using a

2mm silica gel chromatotron plate, eluting with 75% petroleum ether/25% ethyl acetate yielded β-keto ester (**4.21**) as a yellow oil (300mg, 65%): mp 118-121 °C (ethyl acetate/petroleum ether, colourless crystals); FTIR (film) 1791 and 1714cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.31 (t, J=7.1Hz, CH_3), 3.20 (AB_q, J_{AB}=13.2Hz, 1H, C4CH_aPh), 3.28 (AB_q, J_{AB}=18.8Hz, 1H, C4CH_aCO), 3.46 (s, COCH_2CO), 3.52 (AB_q, J_{AB}=13.2Hz, 1H, C4CH_bPh), 4.10 (AB_q, J_{AB}=18.8Hz, 1H, C4CH_bCO), 4.23 (q, J=7.1Hz, CH_2CH$_3$), 4.79 (AB_q, J_{AB}=12.2Hz, 1H, OCH_aPh), 5.05 (AB_q, J_{AB}=12.2Hz, 1H, OCH_bPh), 6.14 (d, J=7.8Hz, (**Ph**)$_2$), 6.38 (s, **H-2**), 6.69 (d, J=7.3Hz, (**Ph**)$_2$), 6.97 (m, (**Ph**)$_2$), 7.08 (m, (**Ph**)$_2$), 7.22 (m, (**Ph**)$_5$), 7.36 (m, (**Ph**)$_2$); ^{13}C NMR ($CDCl_3$) δ 13.98, 41.74, 47.81, 48.81, 61.60, 64.28, 90.39, 127.50, 127.65, 127.81, 127.94, 128.11, 128.93, 128.98, 129.16, 130.73, 134.63, 135.19, 135.52, 152.19, 166.17, 172.74, 200.45; HRMS (M) Found 515.1943 (Calcd for $C_{30}H_{29}NO_7$ 515.1944); $(\alpha)_D^{20}$ = -1 (15.5; CH_2Cl_2). Anal. Calcd for $C_{30}H_{29}NO_7$: C 69.89; H 5.67; N 2.72. Found: C 69.69; H 5.60; N 2.65.

Preparation[E.23] *of Magnesium Diethyl Malonate*

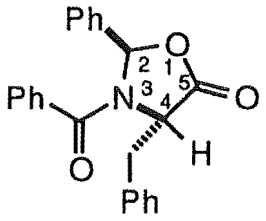
Magnesium ethoxide (314mg, 2.74mmol, 1equiv) was added to ethyl malonate[E.24] (725mg, 5.49mmol, 2equiv) in THF (16mL) and the mixture was stirred at 20 °C for 2h. The solvent was evaporated at 20mm and finally at 1mm to yield magnesium diethyl malonate as a white solid which was used in subsequent steps without further purification.

SECTION E.4.2

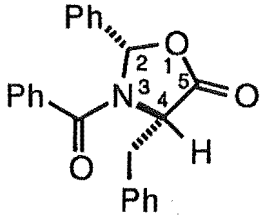
PREPARATION OF BENZOYL OXAZOLIDINONES

(4.22, 4.25-4.26, 4.29-4.30, 4.32-4.33)

Preparation of (2S,4S) 3-Benzoyl-4-benzyl-2-phenyl-1,3-oxozolidin-5-one[E.25] **(4.22):**



4.22

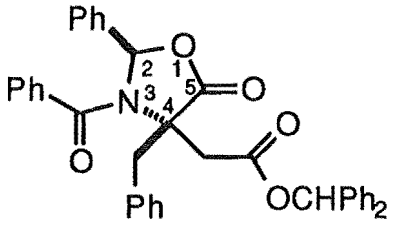


4.23

The Schiff base salt[E.19-E.20] (**4.09**) (16.4g, 0.060mol, 1equiv) of (S)-phenylalanine (**4.08**) in CH_2Cl_2 (90mL) was cooled to -20 °C, benzoyl chloride (10.4mL, 0.089mol, 1.5equiv) was added and the mixture was stirred at -20 °C for 12h and then at 4 °C for 4 days. The mixture was washed with H_2O (100mL), 5% aqueous $NaHCO_3$ solution (100mL), 5% aqueous $NaHSO_3$ solution (100mL) and H_2O (100mL), dried ($MgSO_4$) and the solvent was evaporated to give a residue containing, by ^1H NMR, trans oxazolidinone (**4.22**) and cis oxazolidinone (**4.23**) in a ratio of 7 : 3, respectively. The trans oxazolidinone (**4.22**) was purified by recrystallization (CH_2Cl_2/petroleum ether): Yield 5.6g, 25%; mp 194-196 °C (colourless crystals) (Lit[E.25] 184.3 °C); ^1H NMR ($CDCl_3$) δ 3.39 (bs, 1H, CH_aPh), 3.73 (bs, 1H, CH_bPh), 5.21 (s, **H-4**), 5.83 (s, **H-2**), 6.70-7.40 (m, **(Ph)**$_{15}$); ^{13}C NMR ($CDCl_3$) δ 34.89, 57.76, 91.25, 126.64, 127.72, 128.52, 128.85, 129.82, 129.88, 130.82, 135.22, 136.13, 169.22, 171.23; $(\alpha)_D^{20} = +302°$ (c 1.0; $CHCl_3$).

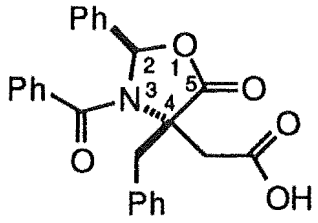
Selected ^1H NMR data for the trans oxazolidinone (**4.23**); ($CDCl_3$) δ 4.92 (bt, **H-4**).

*Preparation of (2S,4R) 3-Benzoyl-4-benzyl-4-(diphenylmethoxycarbonylmethyl)-2-phenyl-1,3-oxozolidin-5-one (**4.25**):*



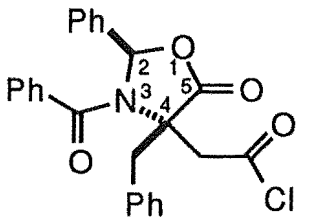
The oxazolidinone (**4.22**) (2.00g, 0.0056mol, 1equiv) was dissolved in THF (200mL) and the solution was cooled to -78 °C. LiHMDS (6.8mL of 1M solution in THF, 0.0068mol, 1.2equiv) was added and the resulting yellow solution was stirred at -78 °C for 30min. $BrCH_2CO_2CHPh_2$[E.21] (1.88g, 0.0062mol, 1.1equiv) was added and the solution was stirred at -78 °C for 2h and was then allowed to warm to 20 °C over 16h. The solution was partitioned between saturated aqueous NH_4Cl solution (150mL) and ether (100mL). The aqueous layer was separated and extracted with ether (100mL). The combined ether extracts were washed with H_2O (2x 50mL), dried (Na_2SO_4) and evaporated to give a yellow solid (1.60g), used subsequently without further purification, which contained, by ^1H NMR, 67% desired oxazolidinone (**4.25**), 22% recovered $BrCH_2CO_2CHPh_2$ and 11% of a compound tentatively assigned as the dimer (**4.33**) (See page 234 for more detail): ^1H NMR ($CDCl_3$) benzhydryl oxazolidinone (**4.25**) δ 3.34 (AB_q, J_{AB}=17.6Hz, 1H,C4C**H**$_a$$CO_2CHPh_2$), 3.41 ($AB_q$, J_{AB}=13.3Hz, 1H, C**H**$_a$Ph), 4.02 (AB_q, J_{AB}=13.3Hz, 1H, C**H**$_b$Ph), 4.16 (AB_q, J_{AB}=17.6Hz, 1H, C**H**$_b$$CO_2CHPh_2$), 5.42 (d, J=7.5Hz, (**Ph**)$_2$), 6.13 (s, **H-2**), 6.42 (m, (**Ph**)$_2$), 6.63 (t, J=7.8Hz, (**Ph**)$_2$), 6.90 (m, (**Ph**)$_3$), 6.92 (s, C**H**Ph_2), 7.10-7.61 (m, (**Ph**)$_{16}$); HRMS (M-91) Found 490.1654 (Calcd for $C_{31}H_{24}NO_5$ 490.1654). ^1H NMR spectroscopy revealed that < 5% of the minor 2S,4S isomer (**4.27**) had formed.

*Preparation of (2S,4R) 3-Benzoyl-4-benzyl-4-carboxymethyl-2-phenyl-1,3-oxozolidin-5-one (**4.29**):*



Benzhydryl oxazolidinone (**4.25**) (1.60g, 0.0027mol, 1equiv) was dissolved in CH_2Cl_2 (70mL) and cooled to 0 °C. TFA (35mL, 0.45mol, 168equiv) was added and the solution was stirred at 0 °C for 5min, then was diluted with CH_2Cl_2 (130mL), washed with H_2O (3x 150mL) and extracted with 5% aqueous $NaHCO_3$ solution. The $NaHCO_3$ extracts were combined, cooled to 0 °C, acidified to pH 1-3 with 1N HCl and extracted with ethyl acetate (3x 200mL). The combined ethyl acetate extracts were dried (Na_2SO_4) and the solvent evaporated to yield oxazolidinone (**4.29**) as a white solid (0.50g, 44%): mp 207.5-211 °C (CH_2Cl_2/pentane, white crystals); FTIR (KBr) 1797, 1725, 1611 and 1595cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.26 (AB_q, J_{AB}=18.0Hz, 1H, CH_aCO_2H), 3.42 (AB_q, J_{AB}=13.4Hz, 1H, CH_aPh), 4.04 (AB_q, J_{AB}=13.4Hz, 1H, CH_bPh), 4.14 (AB_q, J_{AB}=18.0Hz, 1H, CH_bCO_2H), 5.54 (d, J=7.2Hz, **(Ph)**$_2$), 6.45 (s, **H-2**), 6.67 (t, J=7.8Hz, **(Ph)**$_2$), 6.82 (m, **(Ph)**$_2$), 6.96 (m, **(Ph)**$_1$), 7.06 (t, J=7.5Hz, **(Ph)**$_2$), 7.17 (m, **(Ph)**$_1$), 7.42 (m, **(Ph)**$_5$); ^{13}C NMR ($CDCl_3$) δ 38.70, 42.33, 66.14, 91.70, 125.30, 127.80, 127.91, 127.97, 128.34, 129.27, 130.93, 134.27, 135.01, 136.07, 170.32, 172.89, 173.57; HRMS (M) Found 415.1417 (Calcd for $C_{25}H_{21}NO_5$ 415.1420); $(\alpha)_D^{20}$ = +64° (c 0.9; CH_2Cl_2). Anal. Calcd for $C_{25}H_{21}NO_5$: C 72.28; H 5.10; N 3.37. Found: C 71.81; H 5.39; N 3.42.

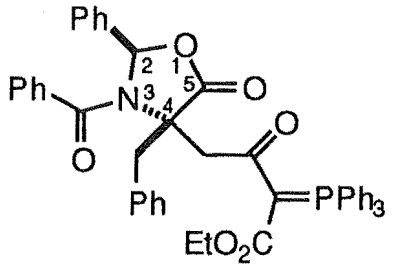
*Preparation of (2S,4R) 3-Benzoyl-4-benzyl-4-chloroformylmethyl-2-phenyl-1,3-oxozolidin-5-one (**4.30**):*



The acid (**4.29**) (66mg, 0.16mmol, 1equiv) was dissolved in CH_2Cl_2 (6mL) and the solution was cooled to 0 °C. Freshly distilled oxalyl chloride (0.69μL, 0.79mmol, 5equiv) and a catalytic quantity of DMF were added. The mixture was stirred at 0 °C for 2h and at 20 °C

for 16h. The solvent was evaporated, more CH_2Cl_2 (2mL) was added and evaporated (repeated 3 times). Final traces of oxalyl chloride were removed at 1mm to yield acid chloride (**4.30**) as a white solid (69mg, quant), which was used in subsequent steps without further purification: ^{1}H NMR ($CDCl_3$) δ 3.38 (AB_q, J_{AB}=13.4Hz, 1H, CH_aPh), 3.76 (AB_q, J_{AB}=19.1Hz, 1H, CH_aCOCl), 3.98 (AB_q, J_{AB}=13.4Hz, 1H, CH_bPh), 4.65 (AB_q, J_{AB}=19.1Hz, 1H, CH_bCOCl), 5.54 (dd, J=1.1, 8.4Hz, **(Ph)**$_2$), 6.39 (s, **H-2**), 6.69 (m, **(Ph)**$_2$), 6.80 (m, **(Ph)**$_2$), 6.98 (m, **(Ph)**$_1$), 7.09 (m, **(Ph)**$_2$), 7.20 (m, **(Ph)**$_1$), 7.36-7.47 (m, **(Ph)**$_5$).

*Preparation of (2S,4R) 3-Benzoyl-4-benzyl-4-(3-ethoxycarbonyl-2-oxo-3-triphenylphosphoranylidenylpropyl)-2-phenyl-1,3-oxozolidin-5-one (**4.26**):*

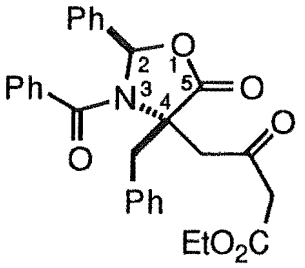


METHOD A: Acid chloride (**4.30**) (69mg, 0.16mmol, 1equiv) was dissolved in CH_2Cl_2 (5mL) and cooled to 0 °C. $Ph_3P{=}CHCO_2Et$[E.03] (111mg, 0.32mmol, 2equiv) was added and the solution was stirred at 0 °C for 1.5h and at 20 °C for 4.5h. The solvent was evaporated and a ^{1}H NMR spectrum of the residue revealed the presence of < 5% of the minor 2S,4S isomer (**4.28**). Purification by radial chromatography using a 1mm silica gel chromatotron plate, eluting with 94% CH_2Cl_2/6% ethyl acetate yielded phosphorane (**4.26**) as a colourless oil (122mg, quant): mp 187-188.5 °C (ethyl acetate/petroleum ether, white crystals); FTIR (KBr) 1787, 1668, 1652 and 1554cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.72 (t, J=7.1Hz, CH_3), 3.41 (AB_q, J_{AB}=13.5Hz, 1H, CH_aPh), 3.77 (m, OCH_2), 4.05 (AB_q, J_{AB}=18.5Hz, 1H, CH_aCO), 4.12 (AB_q, J_{AB}=13.5Hz, 1H, CH_bPh), 4.59 (AB_q, J_{AB}=18.5Hz, 1H, CH_bCO), 5.33 (d, J=7.5Hz, **(Ph)**$_2$), 6.06 (s, **H-2**), 6.38 (d, J=7.5Hz, **(Ph)**$_2$), 6.55 (t, J=7.7Hz **(Ph)**$_2$), 6.85 (t, J=7.7Hz, **(Ph)**$_3$), 7.05 (t, J=7.5Hz, **(Ph)**$_1$), 7.32-7.74 (m, **(Ph)**$_{20}$); ^{31}P NMR ($CDCl_3$) δ 18.3; ^{13}C NMR ($CDCl_3$) δ 13.67, 42.89, 45.89 (d, J=7.1Hz), 58.52, 66.74, 70.84 (d, J=110.6Hz), 90.73, 125.43, 126.25 (d, J=93.2Hz), 127.22, 127.70, 128.01, 128.23, 128.49 (d, J=12.6Hz), 128.69, 128.79, 130.96, 131.70 (d, J=2.9Hz), 133.38 (d, J=10.0Hz), 135.10, 136.40, 137.18, 167.37 (d, J=14.4Hz), 169.02, 174.47, 192.91 (d, J=4.6Hz); HRMS

(FAB, M+1) Found 746.2681 (Calcd for $C_{47}H_{41}NO_6P$ 746.2671). Anal. Calcd for $C_{47}H_{40}NO_6P$: C 75.69; H 5.41; N 1.88. Found: C 75.62; H 5.52; N 1.84.

METHOD B: Oxazolidinone (**4.22**) (30mg, 0.08mmol, 1equiv) was dissolved in THF (10mL) and cooled to -78 °C. LiHMDS (0.09mL, 0.09mmol, 1.1equiv) was added and the resulting yellow solution was stirred at -78 °C for 10min. $BrCH_2COC(PPh_3)CO_2Et$ (39mg, 0.08mmol, 1equiv) was added and the solution was stirred at -78 °C for 2h and was then allowed to warm to 20 °C over 16h. The THF was evaporated and the residue partitioned between saturated aqueous NH_4Cl solution (10mL) and CH_2Cl_2 (10mL). The aqueous layer was separated and extracted with CH_2Cl_2 (2x 10mL). The combined CH_2Cl_2 extracts were dried ($MgSO_4$) and evaporated to give a residue (59mg) containing, by ^1H NMR, 15% phosphorane (**4.26**), 70% recovered $BrCH_2COC(PPh_3)CO_2Et$ and 15% of the compound tentatively assigned as the dimer (**4.33**) (See page 234 for data). Again, < 5% of the minor 2S,4S isomer (**4.28**) was observed by ^1H NMR. The desired phosphorane (**4.26**) was not separated from $BrCH_2COC(PPh_3)CO_2Et$ on silica or diol.

Preparation[E.23] *of (2S,4R) 3-Benzoyl-4-benzyl-4-(3-ethoxycarbonyl-2-oxopropyl)-2-phenyl-1,3-oxozolidin-5-one* (**4.32**):

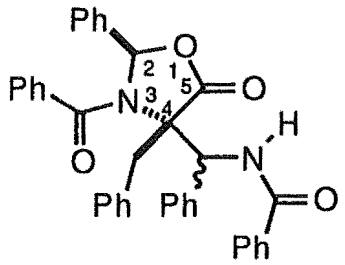


METHOD A: Carbonyl diimidazole (CDI) (23mg, 0.14mmol, 1.1equiv) was added to acid (**4.29**) (50mg, 0.12mmol, 1equiv) in THF (5mL). After stirring at 20 °C for 2h, freshly prepared magnesium diethyl malonate (34mg, 0.12mmol, 1equiv) was added and the mixture was stirred at 20 °C for 19h. The mixture was concentrated to 1mL, diluted with ethyl acetate (5mL) and washed with H_2O (4mL), 5% aqueous $KHSO_4$ solution (4mL), 5% aqueous $NaHCO_3$ solution (4mL) and 10% aqueous NaCl solution (4mL). The organic layer was dried (Na_2SO_4) and the solvent evaporated. Purification by radial chromatography using a 1mm silica gel chromatotron plate, eluting with 70% petroleum ether/30% ethyl

acetate gave β-keto ester (**4.32**) as an oil (91%): mp 103-104 °C (ethyl acetate/petroleum ether); FTIR (film) 1793, 1743, 1716 and 1650cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.27 (t, J=7.1Hz, CH_3), 3.37 (AB_q, J_{AB}= 13.4Hz, 1H, C4CH_aPh), 3.41 (AB_q, J_{AB}=19.0Hz, 1H, C4CH_aCO), 3.56 (AB_q, J_{AB}=15.7Hz, 1H, CH_aCO$_2$Et), 3.61 (AB_q, J_{AB}=15.7Hz, 1H, CH_bCO$_2$Et), 3.99 (AB_q, J_{AB}=13.4Hz, 1H, C4CH_bPh), 4.22 (q, J=7.1Hz, OCH_2), 4.39 (AB_q, J_{AB}=19.0Hz, 1H, C4CH_bCO), 5.54 (dd, J=1.2, 8.5Hz, (**Ph**)$_2$), 6.49 (s, **H-2**), 6.68 (m, (**Ph**)$_2$), 6.78 (m, (**Ph**)$_2$), 6.96 (m, (**Ph**)$_1$), 7.05 (m, (**Ph**)$_2$), 7.17 (m, (**Ph**)$_1$), 7.41 (m, (**Ph**)$_5$); ^{13}C NMR ($CDCl_3$) δ 14.06, 42.25, 48.04, 49.06, 61.90, 65.38, 91.63, 125.34, 127.76, 127.91, 127.96, 128.24, 129.02, 129.23, 129.41, 130.93, 134.45, 135.03, 136.30, 166.14, 169.96, 172.99, 201.31; HRMS (M) Found 485.1807 (Calcd for $C_{29}H_{27}NO_6$ 485.1838); $(\alpha)_D^{20}$ = +67° (c 2.0; CH_2Cl_2). Anal. Calcd for $C_{29}H_{27}NO_6$: C 71.74; H 5.61; N 2.88. Found: C 70.14; H 5.66; N 2.76.

METHOD B: Acid chloride (**4.30**) (52mg, 0.12mmol, 1equiv) and pyridine (24μL, 0.30mmol, 2.5equiv) were dissolved in CH_2Cl_2 (1mL) and cooled to 0 °C. Meldrum's acid (18mg, 0.12mmol 1.03equiv), dissolved in CH_2Cl_2 (1mL), was added to the stirred solution over the period of 105min. The resulting bright yellow solution was stirred at 0 °C for 1h, 20 °C for 1h and then was diluted with CH_2Cl_2 (2mL) and poured into 2N HCl (4mL) containing crushed ice. The organic layer was removed and the aqueous layer was washed with CH_2Cl_2 (2x 2mL). The combined organic extracts were washed with 2N HCl (2x 2mL), dried ($MgSO_4$) and the solvent evaporated to yield an oil (49mg) which was dissolved in ethanol (15mL) and refluxed for 2.5h. The solvent was evaporated and the residue was purified by radial chromatography using a 1mm silica gel chromatotron plate, eluting with 70% petroleum ether/30% ethyl acetate to give β-keto ester (**4.32**) as an oil (12mg, 21%): ^{1}H NMR ($CDCl_3$) as given above.

Benzoyl dimer (**4.33**):

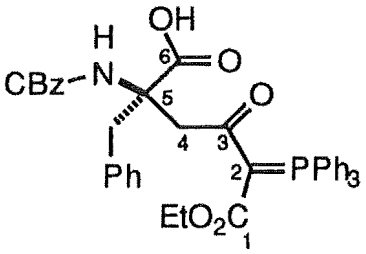


LiHMDS (0.55mL of 1M solution in THF, 0.55mmol, 1.1equiv) was added to oxazolidinone (**4.22**) (180mg, 0.50mmol, 1equiv) dissolved in THF (15mL) at -78 °C. The resulting yellow solution was stirred at -78 °C for 2h and then 20 °C for 16h. The solution was poured onto cold saturated aqueous NH_4Cl solution (15mL) and extracted with ether (2x 15mL). The combined ether extracts were washed with H_2O (15mL), dried ($MgSO_4$) and the solvent was evaporated. Purification by radial chromatography using a 1mm silica gel chromatotron plate, eluting with a gradient of ethyl acetate (1-20%) in CH_2Cl_2 yielded the compound tentatively assigned as the dimer (**4.33**), as a yellow oil (89mg, 62%): mp 237-238 °C (ethyl acetate/petroleum ether, white crystals); FTIR (film) 3350, 1791 and 1667cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.61 (AB_q, J_{AB}=13.9Hz, 1H, C**H**$_a$Ph), 4.46 (AB_q, J_{AB}=13.9Hz, 1H, C**H**$_b$Ph), 4.72 (s, **H-2**), 5.26 (dd, J=1.3, 8.5Hz, (**Ph**)$_2$), 6.22 (d, J=8.8Hz, C**H**PhNH), 6.55 (t, J=7.8Hz, (**Ph**)$_2$), 6.67 (m, (**Ph**)$_2$), 6.86 (m, (**Ph**)$_1$), 6.98 (t, J=7.6Hz, (**Ph**)$_2$), 7.08 (m, (**Ph**)$_1$), 7.42-7.63 (m, (**Ph**)$_{13}$), 8.08 (m, (**Ph**)$_2$), 9.52 (d, J=8.8Hz, N**H**); ^{13}C NMR ($CDCl_3$) δ 30.08, 60.75, 74.63, 90.74, 124.69, 127.46, 127.53, 127.73, 127.94, 128.28, 128.72, 129.02, 129.21, 129.31, 129.40, 131.05, 131.81, 133.57, 133.79, 135.13, 135.72, 137.58, 166.18, 171.52, 172.64; HRMS (FAB, M+1) Found 567.2283 (Calcd for $C_{37}H_{31}N_2O_4$ 567.2284). Anal. Calcd for $C_{37}H_{30}N_2O_4$: C 78.43; H 5.34; N 4.94. Found: C 78.47; H 5.70; N 4.91.

SECTION E.4.3

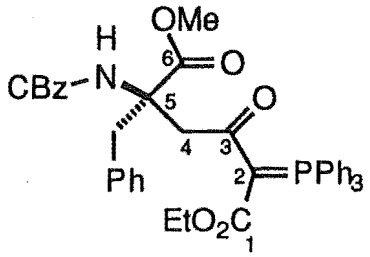
PREPARATION OF ENAMINO ESTERS (**4.01-4.06**) VIA THE INSERTION REACTION

*Preparation of 1-Ethyl (5S) 5-benzyl-5-benzyloxycarbonylamino-3-oxo-2-triphenylphosphoranylidenehexandioate (**4.40**):*



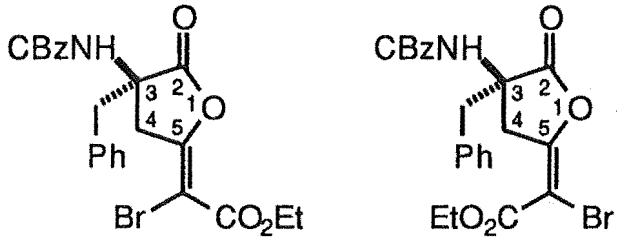
MeOH (48mL) followed by aqueous LiOH (24mL of a 3.33N solution, 79.9mmol, 103equiv) were added to oxazolidinone (**4.15**) (600mg, 0.77mmol, 1equiv) dissolved in THF (48mL). The mixture was refluxed for 4h, cooled to 0 °C and acidified to pH 1-3 with 2N HCl. The THF was evaporated and the remaining solution was extracted with ethyl acetate (3x 50mL). The combined ethyl acetate extracts were dried ($MgSO_4$) and the solvent was evaporated at 20mm, and finally at 1mm for 16h, to yield keto acid phosphorane (**4.40**), as a white solid (530mg, quant), which was used in subsequent steps without further purification: FTIR (KBr) 3404, 1790, 1715, 1667 and 1559cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.74 (t, J=7.1Hz, CH_3), 2.86 (AB_q, J_{AB}=13.5Hz, 1H, CCH_aPh), 2.94 (AB_q, J_{AB}=17.6Hz, 1H, CCH_aCO), 3.52 (AB_q, J_{AB}=13.5Hz, 1H, CCH_bPh), 3.83 (m, CH_2CH_3), 4.97 (AB_q, J_{AB}=12.2Hz, 1H, OCH_aPh), 5.03 (AB_q, J_{AB}=17.6Hz, 1H, CCH_bCO), 5.29 (AB_q, J_{AB}=12.2Hz, 1H, OCH_bPh), 6.08 (s, NH), 6.87 (d, J=7.8Hz, $(Ph)_2$), 7.09 (m, $(Ph)_3$), 7.33-7.51 (m, $(Ph)_{11}$), 7.57 (m, $(Ph)_3$), 7.69 (m, $(Ph)_6$); ^{31}P NMR ($CDCl_3$) δ 18.6; ^{13}C NMR ($CDCl_3$) δ 13.45, 37.55, 41.49 (d, J=6.1Hz), 59.85, 62.64, 65.94, 124.44 (d, J=93.7Hz), 126.62, 127.94, 128.05, 128.31, 128.59, 128.82 (d, J=13.1Hz), 129.70, 132.40 (d, J=2.1Hz), 133.06 (d, J=10.0Hz), 135.52, 136.86, 154.26, 166.55 (d, J=13.1Hz), 173.94, 192.79 (d, J=4.0Hz); HRMS (FAB, M+1) Found 688.2461 (Calcd for $C_{41}H_{39}NO_7P$ 688.2464).

*Preparation of 1-Ethyl, 6-methyl (5S) 5-benzyl-5-benzyloxycarbonylamino-3-oxo-2-triphenylphosphoranylidenehexandioate (**4.41**):*



Keto-acid phosphorane (**4.40**) (89mg, 0.13mmol) was dissolved in THF (1mL) and treated with an excess of freshly distilled CH_2N_2 in ether. The excess CH_2N_2 was allowed to evaporate at 20 °C over 16h and the residue was purified by radial chromatography using a 1mm silica gel chromatotron plate, eluting with a gradient of ethyl acetate (25-50%) in petroleum ether to give phosphorane (**4.41**) as a white solid (75mg, 82%): mp 181-185 °C (ethyl acetate/petroleum ether, white crystals): FTIR (KBr) 3419, 1722, 1666 and 1555cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.69 (t, J=7.1Hz, CH_2CH_3), 3.29 (AB_q, J_{AB}=13.6Hz, 1H, CCH_aPh), 3.49 (s, OCH_3), 3.56 (AB_q, J_{AB}=13.6Hz, 1H, CCH_bPh), 3.74 (m, 4H, CH_2CH_3 and CCH_2CO), 5.11 (AB_q, J_{AB}=12.7Hz, 1H, OCH_aPh), 5.21 (AB_q, J_{AB}=12.7Hz, 1H, OCH_bPh), 6.22 (s, NH), 6.97 (m, $(Ph)_2$), 7.14 (m, $(Ph)_3$), 7.37 (m, $(Ph)_{11}$), 7.47 (m, $(Ph)_3$), 7.61 (m, $(Ph)_6$); ^{31}P NMR ($CDCl_3$) δ 18.0; ^{13}C NMR ($CDCl_3$) δ 13.73, 40.82, 45.56 (d, J=6.1Hz), 52.04, 58.56, 62.13, 65.79, 71.79 (d, J=110.8Hz), 126.27 (d, J=93.7Hz), 126.50, 127.85, 127.86 (d, J=14.1Hz), 128.33, 128.50, 130.33, 131.59 (d, J=3.0Hz), 132.22 (d, J=10.0Hz), 136.21, 137.15, 154.75, 167.57 (d, J=15.1Hz), 173.15, 193.11 (d, J=4.0Hz); HRMS (FAB, M+1) Found 702.2618 (Calcd for $C_{42}H_{41}NO_7P$ 702.2621); $(\alpha)_D^{20}$ = +4° (c 3.1; CH_2Cl_2). Anal. Calcd for $C_{42}H_{40}NO_7P$: C 71.89; H 5.75; N 2.00. Found: C 71.56; H 5.42; N 1.86.

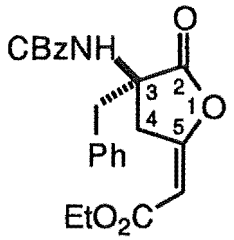
*Preparation of (3S) (E)- and (Z)-3-benzyl-3-benzyloxycarbonylamino-5-bromoethoxycarbonylmethylidene-2-tetrahydrofuranone (**4.42E** and **4.42Z**):*



4.42E **4.42Z**

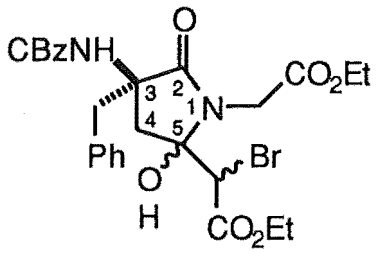
Triethylamine (58μL, 0.44mmol, 1equiv) followed by Br_2 (22μL, 0.44mmol, 1equiv) were added to keto acid phosphorane (**4.40**) (300mg, 0.44mmol, 1equiv), dissolved in CH_2Cl_2 (30mL), at 0 °C. The solution was stirred at 0 °C for 20min and then at 20 °C for 30min. The solvent was evaporated to give crude E- and Z-bromo enollactones (**4.42E** and **4.42Z**, respectively) in the ratio 46% E : 54% Z, by ^1H NMR. Purification by radial chromatography using a 2mm silica gel chromatotron plate, eluting with CH_2Cl_2 yielded Z-enollactone (**4.42Z**) (78mg, 37%), as a white solid, which was used in subsequent steps without further purification: FTIR (film) 3335, 1825, 1704, 1638 and 1524cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.32 (t, J=7.1Hz, C**H**$_3$), 2.98 (AB$_q$, J_{AB}=13.2Hz, 1H, C3C**H**$_a$Ph), 3.14 (AB$_q$, J_{AB}=13.2Hz, 1H, C3C**H**$_b$Ph), 3.49 (AB$_q$, J_{AB}=19.1Hz, 1H, (**H-4**)$_a$), 3.80 (AB$_q$, J_{AB}=19.1Hz, 1H, (**H-4**)$_b$), 4.22 (m, C**H**$_2$CH$_3$), 5.09 (m, OC**H**$_2$Ph), 5.40 var (s, N**H**), 7.17 (m, (**Ph**)$_2$), 7.34 (m, (**Ph**)$_8$); ^{13}C NMR ($CDCl_3$) δ 14.12, 39.07, 42.50, 60.38, 62.07, 67.68, 90.68, 128.33, 128.42, 128.50, 128.61, 129.04, 129.92, 131.63, 135.35, 154.95, 159.71, 162.66, 172.69; HRMS (M) Found 489.0614 (Calcd for $C_{23}H_{22}{}^{81}BrNO_6$ 489.0611); $(\alpha)_D{}^{20}$ = -2° (c 1.5; CH_2Cl_2). Anal. Calcd for $C_{23}H_{22}BrNO_6$: C 56.57; H 4.54; N 2.87. Found: C 56.75; H 4.67; N 2.53. Further elution with CH_2Cl_2 yielded E-enollactone (**4.42E**) (66mg, 31%), as a white solid, which was used in subsequent steps without further purification: FTIR (film) 3337, 1823, 1712, 1642 and 1523cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.29 (t, J=7.1Hz, C**H**$_3$), 3.03 (AB$_q$, J_{AB}=13.2Hz, 1H, C3C**H**$_a$Ph), 3.15 (AB$_q$, J_{AB}=13.2Hz, 1H, C3C**H**$_b$Ph), 3.37 (m, (**H-4**)$_2$), 4.22 (q, J=7.1Hz, C**H**$_2$CH$_3$), 5.11 (m, OC**H**$_2$Ph), 5.40 (s, N**H**), 7.18 (m, (**Ph**)$_2$), 7.34 (m, (**Ph**)$_8$); ^{13}C NMR ($CDCl_3$) δ 14.09, 40.22, 42.49, 59.94, 62.20, 67.68, 94.60, 128.37, 128.53, 128.62, 129.08, 129.88, 131.71, 135.34, 154.93, 155.25, 160.72, 173.86; HRMS (M) Found 489.0608 (Calcd for $C_{23}H_{22}{}^{81}BrNO_6$ 489.0611); $(\alpha)_D{}^{20}$ = +7° (c 0.9; CH_2Cl_2). Anal. Calcd for $C_{23}H_{22}BrNO_6$: C 56.57; H 4.54; N 2.87. Found: C 56.81; H 4.60; N 2.83.

*Preparation of (3S) (E)-3-benzyl-3-benzyloxycarbonylamino-5-ethoxycarbonylmethylidene-2-tetrahydrofuranone (**4.43**):*



Keto acid phosphorane (**4.40**) (62mg, 0.090mmol) was dissolved in THF (7mL) and refluxed for 6h. The solvent was evaporated and purification by radial chromatography using a 1mm silica gel chromatotron plate, eluting with 97% CH_2Cl_2/3% ethyl acetate yielded enollactone (**4.43**), as a pale yellow oil (37mg, 73%), which crystallized on standing at 4 °C: mp 106-108 °C (ethyl acetate/petroleum ether, white crystals); FTIR (KBr) 3391, 1807, 1712 and 1526cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.27 (t, J=7.1Hz, C**H**$_3$), 2.99 (AB$_q$, J_{AB}=13.2Hz, 1H, C3C**H**$_a$Ph), 3.14 (AB$_q$, J_{AB}=13.2Hz, 1H, C3C**H**$_b$Ph), 3.50 (AB$_q$, J_{AB}=19.1Hz, 1H, (**H-4**)$_a$), 3.82 (AB$_q$, J_{AB}=19.1Hz, 1H, (**H-4**)$_b$), 4.15 (m, C**H**$_2$CH$_3$), 5.10 (m, OC**H**$_2$Ph), 5.34 (s, =C**H**), 5.46 (s, N**H**), 7.18 (m, (**Ph**)$_2$), 7.32 (m, (**Ph**)$_8$); ^{13}C NMR ($CDCl_3$) δ 14.26, 37.30, 42.53, 59.52, 60.10, 67.68, 97.87, 128.33, 128.41, 128.53, 128.63, 129.08, 130.08, 131.95, 135.42, 154.93, 163.62, 166.25, 173.72; HRMS (FAB, M+1) Found 410.1604 (Calcd for $C_{23}H_{24}NO_6$ 410.1604); $(\alpha)_D^{20}$ = -13° (c 0.6; CH_2Cl_2). Anal. Calcd for $C_{23}H_{23}NO_6$: C 67.47; H 5.66; N 3.42. Found: C 67.70; H 5.41; N 3.51.

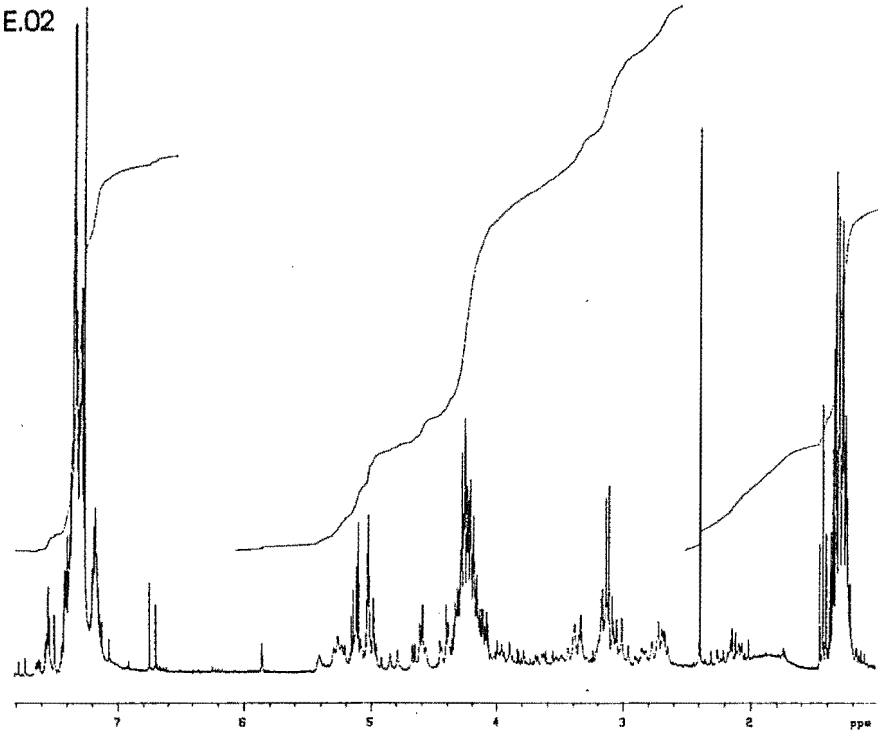
*Preparation of (3S,5R and/or S, 5'R and/or S) 3-Benzyl-3-benzyloxycarbonylamino-5-bromo(ethoxycarbonyl)methyl-1-ethoxycarbonylmethyl-5-hydroxy-2-pyrrolidinone (**4.44**):*



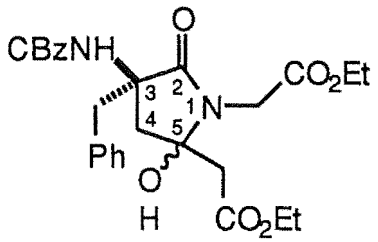
Glycine ethylester hydrochloride (60mg, 0.43mmol, 3equiv) and triethylamine (57μL, 0.43mmol, 3equiv) were added to E- or Z-bromo enollactone (**4.42E** or **4.42Z**, respectively) (70mg, 0.14mmol, 1equiv), dissolved in CH_2Cl_2 (35mL). The mixture was stirred for 16h, washed with H_2O (35mL), dried ($MgSO_4$) and the solvent evaporated to yield bromo

hydroxy lactam (**4.44**) as an orange oil (250mg, quant) which was used in subsequent steps without further purification. Bromo hydroxy lactam (**4.44**) was obtained as a mixture of diastereoisomers: ^{1}H NMR ($CDCl_3$) shown below (Spectrum E.02):

Spectrum E.02



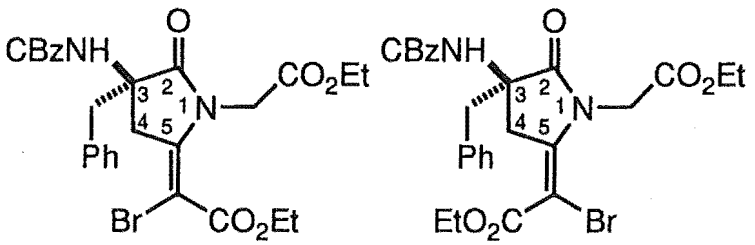
*Preparation of (3S,5R) and (3S,5S) 3-Benzyl-3-benzyloxycarbonylamino-1-ethoxycarbonylmethyl-5-ethoxycarbonylmethyl-5-hydroxy-2-pyrrolidinone (**4.45**):*



Glycine ethylester hydrochloride (75mg, 0.54mmol, 2equiv) and triethylamine (71μL, 0.54mmol, 2equiv) were added to protio enollactone (**4.43**) (110mg, 0.27mmol, 1equiv), dissolved in CH_2Cl_2 (40mL). The mixture was stirred for 16h, washed with H_2O (40mL), dried ($MgSO_4$) and the solvent evaporated to yield protio hydroxy lactam (**4.45**) as a yellow oil (107mg, 78%), which was used in subsequent steps without further purification: FTIR (film) 3412 and 1713cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.25 (t, J=7.3Hz, C**H$_3$**), 1.30 (t, J=7.3Hz, C**H$_3$**), 2.78 (m, 4H, C5C**H$_2$**CO_2Etand (**H-4**)$_2$), 3.14 (AB_q, J_{AB}=13.7Hz, 1H, C3C**H$_a$**Ph), 3.31 (AB_q, J_{AB}=13.7Hz, 1H, C3C**H$_b$**Ph), 4.01 (AB_q, J_{AB}=17.6Hz, 1H, NC**H$_a$**), 4.12 (q, J=7.3Hz, C**H$_2$**CH_3), 4.23 (q, J=7.3Hz,

CH_2CH_3), 4.26 (AB_q, J_{AB}=17.6Hz, 1H, NCH_b), 5.03 (AB_q, J_{AB}=12.3Hz, 1H, OCH_aPh), 5.09 (AB_q, J_{AB}=12.3Hz, 1H, OCH_bPh), 5.30 (s, **NH**), 7.22 (m, $(Ph)_2$), 7.34 (m, $(Ph)_8$); ^1H NMR ($CDCl_3$) also indicated the presence of another diastereoisomer; 12%, δ 5.42 (s, **NH**); ^{13}C NMR ($CDCl_3$) selected resonances for both diastereoisomers δ 13.95, 13.98, 14.02, 40.70, 41.37, 42.52, 42.80, 42.85, 43.59, 43.64, 59.64, 60.16, 60.65, 61.19, 61.79, 66.69. 67.36, 76.02, 86.38, 126.46, 127.38, 127.47, 127.53, 128.11, 128.19, 128.35, 128.43, 128.50, 128.65, 128.70, 130.44, 134.87, 136.03, 154.77, 155.78, 168.45, 169.06, 169.62, 170.11, 173.68, 174.21; LRMS (CI) 513 (5), 495 (13), 403 (22), 108 (14), 91 (100).

*Preparation of (3S) (E)- and (Z)-3-Benzyl-3-benzyloxycarbonylamino-5-bromoethoxycarbonylmethylidene-1-ethoxycarbonylmethyl-2-pyrrolidinone (**4.01E** and **4.01Z**):*

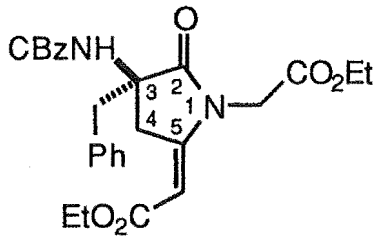


4.01E **4.01Z**

Bromo hydroxy lactam (**4.44**) (0.14mmol, 1equiv) and PTSA (14mg) dissolved in 1, 2-dichloroethane (35mL) were refluxed, with azeotropic removal of H_2O, for 3.5h. The solvent was evaporated and purification by radial chromatography using a 1mm silica gel chromatotron plate, eluting with 96% CH_2Cl_2/4% ethyl acetate yielded a pale yellow oil (52mg, 65%) which contained E- and Z-bromo enamino esters (**4.01E** and **4.01Z**, respectively) in the ratio 15% E : 85% Z, by ^1H NMR: FTIR (film) 3351, 1747, 1713 and 1602cm^{-1}; ^1H NMR ($CDCl_3$) Z isomer (**4.01Z**) from mixture δ 1.29 (t, J=7.1Hz, CH_3), 1.33 (t, J=7.1Hz, CH_3), 3.04 (AB_q, J_{AB}=13.2Hz, 1H, $C3CH_aPh$), 3.09 (AB_q, J_{AB}=13.2Hz, 1H, $C3CH_bPh$), 3.42 (AB_q, J_{AB}=17.3Hz, 1H, $(H\text{-}4)_a$), 3.92 (AB_q, J_{AB}=17.3Hz, $(H\text{-}4)_b$), 4.25 (m, 4H, 2x CH_2CH_3), 4.76 (AB_q, J_{AB}=18.4Hz, 1H, NCH_a), 4.83 (AB_q, J_{AB}=18.4Hz, 1H, NCH_b), 5.08 (m, 3H, OCH_2Ph and =CH), 5.29 (s, **NH**), 7.13 (m, $(Ph)_2$), 7.34 (m, $(Ph)_8$); ^1H NMR ($CDCl_3$) E isomer (**4.01E**) from mixture δ 5.36 (s, **NH**); ^{13}C NMR ($CDCl_3$) Z isomer (**4.01Z**) from mixture δ 14.08, 14.18, 40.31, 42.49, 44.89, 59.09, 61.79, 61.91, 67.16, 98.67, 127.85, 128.34, 128.43, 128.54, 128.70, 130.10, 133.25, 135.74, 148.00, 154.75, 163.55, 167.69, 176.52; HRMS (CI, M+1) Found 575.1125 (Calcd for

$C_{27}H_{30}{}^{81}BrN_2O_7$ 575.1217), Found 573.1238 (Calcd for $C_{27}H_{30}{}^{79}BrN_2O_7$ 573.1237). Further elution with 70% petroleum ether/30% ethyl acetate yielded imide (**4.46**) as a pale yellow oil (8mg, 13%): IR (film) 3350, 1790, 1715, 1630 and 1520cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, J=7.1Hz, C**H$_3$**), 3.04 (m, 4H, C3C**H$_2$**Ph and (**H-4**)$_2$), 4.22 (m, 4H, C**H$_2$**CH$_3$ and NC**H$_2$**), 5.04 (AB_q, J_{AB}=12.2Hz, 1H, OC**H$_a$**Ph), 5.11 (AB_q, J_{AB}=12.2Hz, 1H, OC**H$_b$**Ph), 5.34 (s, N**H**), 7.17 (m, (**Ph**)$_2$), 7.34 (m, (**Ph**)$_8$); ^{13}C NMR ($CDCl_3$) δ 14.06, 39.51, 39.82, 42.62, 60.17, 61.96, 67.42, 128.13, 128.38, 128.49, 128.63, 129.05, 130.06, 132.96, 135.56, 154.97, 166.56, 172.90, 176.79; HRMS (M) Found 424.1629 (Calcd for $C_{23}H_{24}N_2O_6$ 424.1634). The yields and isomer ratios of enamino esters (**4.01E** and **4.01Z**) were the same when the reaction was carried out with E-bromo enollactone (**4.42E**) and Z-bromo enollactone (**4.42Z**)

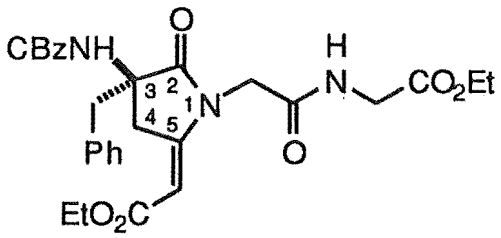
*Preparation of (3S) (E)-3-Benzyl-3-benzyloxycarbonylamino-1-ethoxycarbonylmethyl-5-ethoxycarbonylmethylidene-2-pyrrolidinone (**4.02**):*



Protio hydroxy lactam (**4.45**) (100mg, 0.20mmol, 1equiv) and PTSA (4mg) dissolved in 1, 2-dichloroethane (35mL) were refluxed, with azeotropic removal of H_2O, for 3h. After cooling to 20 °C the solution was washed with H_2O (10mL), dried ($MgSO_4$) and the solvent was evaporated. Purification by radial chromatography using a 1mm silica gel chromatotron plate, eluting with 94% CH_2Cl_2/6% ethyl acetate yielded enamino ester (**4.02**) as a colourless oil (65mg, 68%): FTIR (KBr) 3345, 1745, 1709, 1630 and 1520cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, J=7.1Hz, C**H$_3$**), 1.28 (t, J=7.1Hz, C**H$_3$**), 3.05 (m, C3C**H$_2$**Ph), 3.38 (AB_q, J_{AB}=18.6Hz, 1H, (**H-4**)$_a$), 3.89 (AB_q, J_{AB}=18.6Hz, 1H, (**H-4**)$_b$), 4.09 (AB_q, J_{AB}=17.6Hz, 1H, NC**H$_a$**), 4.15 (m, C**H$_2$**CH$_3$), 4.22 (q, J=7.1Hz, C**H$_2$**CH$_3$), 4.43 (AB_q, J_{AB}=17.6Hz, 1H, NC**H$_b$**), 4.99 (s, =C**H**), 5.01 (AB_q, J_{AB}=11.8Hz, 1H, OC**H$_a$**Ph), 5.10 (AB_q, J_{AB}=11.8Hz, 1H, OC**H$_b$**Ph), 5.27 (s, N**H**), 7.17 (m, (**Ph**)$_2$), 7.31 (m, (**Ph**)$_8$); ^{13}C NMR ($CDCl_3$) δ 14.04, 14.34, 36.90, 41.94, 42.46, 59.33, 59.73, 61.93, 67.14, 92.86, 127.75, 128.30, 128.39, 128.51, 128.75, 130.12, 133.32, 135.74, 154.65, 154.81, 166.38, 166.52, 175.56; HRMS (M) Found 494.2065 (Calcd for $C_{27}H_{30}N_2O_7$ 494.2053); $(\alpha)_D{}^{20}$ =

+20° (c 2.3; CH_2Cl_2). Anal. Calcd for $C_{27}H_{30}N_2O_7$: C 65.57; H 6.11; N 5.66. Found: C 65.87; H 6.39; N 5.37.

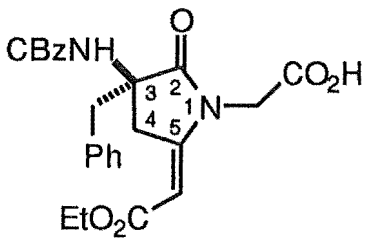
*(3S) (E)-3-benzyl-3-benzyloxycarbonylamino-5-ethoxycarbonylmethylidene-1-(N-ethoxycarbonylmethyl)-carbamoylmethyl-2-pyrrolidinone (**4.03**):*



METHOD A: Glycylglycine ethylester hydrochloride (78mg, 0.40mmol, 5.4equiv) and triethylamine (52μL, 0.40mol, 5.4equiv) were added to enollactone (**4.43**) (30mg, 0.073mmol, 1equiv) dissolved in 1, 2-dichloroethane (10mL) and the mixture was refluxed, with azeotropic removal of H_2O, for 44h. After cooling to 20 °C, the mixture was washed with H_2O (10mL), dried ($MgSO_4$) and the solvent evaporated to give a yellow oil (43mg) which was dissolved in 1, 2-dichloroethane (10mL). PTSA (16mg) was added and the solution was refluxed, with azeotropic removal of H_2O, for 4h. The solvent was evaporated and purification by radial chromatography using a 1mm silica gel chromatotron plate, eluting with 80% CH_2Cl_2/20% ethyl acetate yielded enamino ester (**4.03**) as a colourless oil (26mg, 64%): FTIR (film) 3339, 1748, 1694, 1633 and 1538cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.21 (t, J=7.1Hz, C**H**$_3$), 1.25 (t, J=7.1Hz, C**H**$_3$), 2.97 (AB$_q$, J_{AB}=13.2Hz, 1H, C3C**H**$_a$Ph), 3.11 (AB$_q$, J_{AB}=13.2Hz, 1H, C3C**H**$_b$Ph), 3.37 (dd, J=2.0, 19.1Hz, 1H, (**H-4**)$_a$), 3.65 (AB$_q$, J_{AB}=17.1Hz, 1H, NC**H**$_a$), 3.79 (dd, J=1.5, 19.1Hz, 1H, (**H-4**)$_b$), 3.84 (dd, J=5.9, 17.3Hz, 1H, NC**H**$_a$), 4.02 (dd, J=5.9, 17.3Hz, 1H, NHC**H**$_b$), 4.12 (q, J=7.1Hz, C**H**$_2$CH$_3$), 4.14 (q, J=7.1Hz, C**H**$_2$CH$_3$), 4.67 (AB$_q$, J_{AB}=17.1Hz, 1H, NC**H**$_b$), 5.02 (s, OC**H**$_2$Ph), 5.11 (s, =C**H**), 5.42 (s, CBzN**H**), 7.19 (m, (**Ph**)$_2$), 7.29 (m, (**Ph**)$_8$), 7.43 (bt, N**H**CH$_2$); ^{13}C NMR ($CDCl_3$) δ 14.05, 14.26, 36.87, 41.37, 42.22, 44.08, 59.10, 59.77, 61.15, 67.69, 93.42, 128.10, 128.19, 128.53, 128.65, 128.97, 130.00, 132.51, 135.31, 153.87, 155.45, 166.10, 166.68, 168.88, 175.53; HRMS (M) Found 551.2258 (Calcd for $C_{29}H_{33}N_3O_8$ 551.2268); $(\alpha)_D^{20}$ = +4° (c 0.8; CH_2Cl_2).

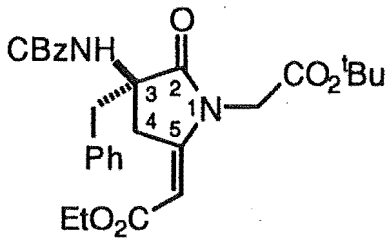
METHOD B: Acid (**4.05**) (0.035mmol, 1equiv), DCC (7mg, 0.035mmol, 1equiv), glycine ethylester hydrochloride (5mg, 0.040mmol, 1.1equiv) and triethylamine (5μL, 0.040mmol, 1.1equiv) in CH_2Cl_2 (2mL) were stirred for 16h at 20 °C. The mixture was diluted with CH_2Cl_2 (5mL), washed with H_2O (7mL), dried ($MgSO_4$) and the solvent evaporated. Purification by radial chromatography using a 1mm silica gel chromatotron plate, eluting with 80% ethyl acetate/20% CH_2Cl_2 yielded a white solid (15mg) which contained enamino ester (**4.03**) (^{1}H NMR ($CDCl_3$) as given above), DCC and DCC by-products.

*Preparation of (3S) (E)-3-Benzyl-3-benzyloxycarbonylamino-1-carboxymethyl-5-ethoxycarbonylmethylidene-2-pyrrolidinone (**4.05**):*



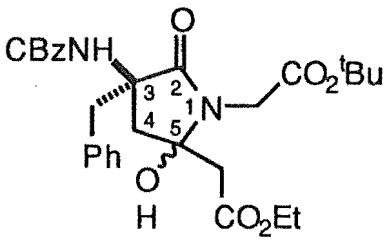
Tert-butyl enamino ester (**4.04**) (0.039mmol, 1equiv) and PTSA (2mg) in benzene (10mL) were refluxed, with azeotropic removal of H_2O, for 3h. Evaporation of the solvent yielded a brown oil (23mg), used subsequently without further purification, containing enamino ester (**4.05**): ^{1}H NMR ($CDCl_3$) δ 1.27 (t, J=7.1Hz, C**H**$_3$), 3.01 (AB$_q$, J_{AB}=13.2Hz, 1H, C3C**H**$_a$Ph), 3.07 (AB$_q$, J_{AB}=13.2Hz, 1H, C3C**H**$_b$Ph), 3.37 (AB$_q$, J_{AB}=18.6Hz, 1H, (**H-4**)$_a$), 3.84 (AB$_q$, J_{AB}=18.6Hz, 1H, (**H-4**)$_b$), 4.15 (m, C**H**$_2$CH$_3$), 4.23 (AB$_q$, J_{AB}=17.5Hz, 1H, NC**H**$_a$), 4.36 (AB$_q$, J_{AB}=17.5Hz, 1H, NC**H**$_b$), 5.03 (AB$_q$, J_{AB}=12.2Hz, 1H, OC**H**$_a$Ph), 5.06 (s, =C**H**), 5.07 (AB$_q$, J_{AB}=12.2Hz, 1H, OC**H**$_b$Ph), 5.38 (s, N**H**), 7.17 (m, (**Ph**)$_2$), 7.37 (m, (**Ph**)$_8$); HRMS (M) Found 466.1737 (Calcd for $C_{25}H_{26}N_2O_7$ 466.1740).

*Preparation of (3S) (E)-3-Benzyl-3-benzyloxycarbonylamino-5-ethoxycarbonylmethylidene-1-(tert-butoxycarbonyl)methyl-2-pyrrolidinone (**4.04**):*



Hydroxy lactam (**4.47**) (21mg, 0.039mmol) and PTSA (2mg) were dissolved in 1, 2-dichloroethane (10mL) and refluxed, with azeotropic removal of H_2O, for 3h. Evaporation of the solvent yielded a beige oil (22mg), used subsequently without further purification, containing enamino ester (**4.04**): ^{1}H NMR ($CDCl_3$) δ 1.27 (t, J=7.1Hz, $CH_2C\mathbf{H_3}$), 1.47 (s, $C(C\mathbf{H_3})_3$), 3.05 (m, $C3C\mathbf{H_2}Ph$), 3.37 (AB_q, J_{AB}=17.6Hz, 1H, (**H-4**)$_a$), 3.82-4.31 (m, 5H, $C\mathbf{H_2}CH_3$, (**H-4**)$_b$ and $NC\mathbf{H_2}$), 5.08 (m, $OC\mathbf{H_2}Ph$), 5.29 (s, =C**H**), 5.38 (s, N**H**), 7.17 (m, (**Ph**)$_2$), 7.31 (m, (**Ph**)$_8$).

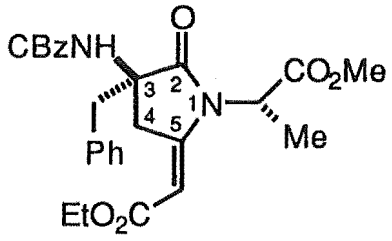
*Preparation of (3S,5R) and (3S,5S) 3-Benzyl-3-benzyloxycarbonylamino-5-ethoxycarbonylmethyl-5-hydroxy-1-(tert-butoxycarbonylmethyl)-2-pyrrolidinone (**4.47**):*



Tert-butyl glycine hydrochloride (13mg, 0.078mmol, 2equiv) and triethylamine (10μL, 0.078mmol, 2equiv) were added to enollactone (**4.43**) (16mg, 0.039mmol, 1equiv) dissolved in CH_2Cl_2 (15 mL). The mixture was stirred for 16h at 20 °C, washed with H_2O (15mL), dried ($MgSO_4$) and the solvent evaporated to yield hydroxy lactam (**4.47**) as a colourless oil (21mg, 100%), which was used in subsequent steps without further purification: FTIR (film) 3412 and 1711cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.25 (t, J=7.1Hz, $CH_2C\mathbf{H_3}$), 1.49 (s, $C(C\mathbf{H_3})_3$), 2.78 (m, 4H, (**H-4**)$_2$ and $C5C\mathbf{H_2}CO_2Et$), 3.15 (AB_q, J_{AB}=13.7Hz, 1H, $C3C\mathbf{H_a}Ph$), 3.32 (AB_q, J_{AB}=13.7Hz, 1H, $C3C\mathbf{H_b}Ph$), 3.92 (AB_q, J_{AB}=10.8Hz, 1H, $NC\mathbf{H_a}$), 4.17 (m, $C\mathbf{H_2}CH_3$), 4.22 (AB_q, J_{AB}=10.8Hz, 1H, $NC\mathbf{H_b}$), 5.02 (AB_q, J_{AB}=12.2Hz, 1H, $OC\mathbf{H_a}Ph$), 5.09 (AB_q, J_{AB}=12.2Hz, 1H, $OC\mathbf{H_b}Ph$), 5.30 (s, N**H**), 7.20 (m, (**Ph**)$_2$), 7.34 (m, (**Ph**)$_8$); ^{1}H NMR ($CDCl_3$) also indicated the

presence of another diastereoisomer; 11%, δ 5.41 (s, NH); ^{13}C NMR ($CDCl_3$) δ 14.04, 27.96, 28.03 (minor diastereoisomer), 42.40, 42.61, 43.81, 60.21, 61.21, 66.71, 82.95, 86.45, 127.47, 128.18, 128.50, 128.66, 130.50, 135.03, 136.14, 154.82, 169.14, 170.06, 174.20; HRMS (M-18) Found 522.2375 (Calcd for $C_{29}H_{34}N_2O_8$ 522.2368).

*(3S,1'S) (E)-3-Benzyl-3-benzyloxycarbonylamino-1-ethoxycarbonylethyl-5-methoxycarbonylmethylidene-2-pyrrolidinone (**4.06**):*

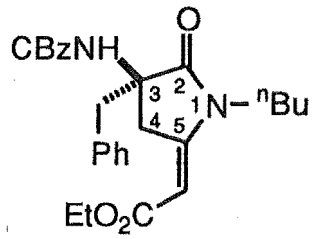


(S)-alanine methylester hydrochloride (189mg, 1.36mmol, 15equiv) and triethylamine (179μL, 1.36mmol, 15equiv) were added to enollactone (**4.43**) (37mg, 0.090mmol, 1equiv) in 1, 2-dichloroethane (25mL) and the mixture was refluxed, with azeotropic removal of H_2O, for 43h. The solvent was evaporated and purification by radial chromatography using a 1mm silica gel chromatotron plate, eluting with 95% CH_2Cl_2/5% ethyl acetate yielded enamino ester (**4.06**) as a yellow oil (35mg, 78%): FTIR (film) 3341, 1743, 1712, 1625 and 1522cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.27 (t, J=7.1Hz, CH_2CH_3), 1.47 (d, J=7.3Hz, NCH**CH$_3$**), 2.98 (AB_q, J_{AB}=13.2Hz, 1H, C3C**H$_a$**Ph), 3.07 (AB_q, J_{AB}=13.2Hz, 1H, C3C**H$_b$**Ph), 3.37 (AB_q, J_{AB}=18.6Hz, 1H, **(H-4)$_a$**), 3.67 (s, O**CH$_3$**), 3.71 (AB_q, J_{AB}=18.6Hz, 1H, **(H-4)$_b$**), 3.87 (dd, J=1.2, 18.3Hz, NC**H**), 4.15 (m, CH_2CH_3), 5.01 (m, 3H, O**CH$_2$**Ph and =C**H**), 5.27 (bs, N**H**), 7.17 (m, **(Ph)$_2$**), 7.33 (m, **(Ph)$_8$**); ^{13}C NMR ($CDCl_3$) δ 12.71, 14.34, 36.99, 42.37, 49.45, 52.72, 59.05, 59.73, 67.12, 93.70, 127.69, 128.22, 128.31, 128.55, 128.78, 130.30, 133.09, 135.75, 153.42, 154.73, 166.64, 169.76, 175.36; HRMS (M+K) Found 533.1692 (Calcd for $C_{27}H_{30}N_2O_7K$ 533.1690); $(\alpha)_D^{20}$ = -17° (c 1.0; CH_2Cl_2). The ^{13}C NMR spectrum indicated the presence of < 5% of another diastereoisomer.

SECTION E.4.4

PREPARATION OF ENAMINO ESTERS (**4.02, 4.07**) VIA THE β-KETO ESTER ROUTE

*Preparation of 1-Butyl (3S)-(E)-3-benzyl-3-benzyloxycarbonylamino-5-ethoxycarbonylmethylidene-2-pyrrolidinone (**4.07**):*



$TiCl_4$ (4μL, 0.037mmol, 0.5equiv) was added to β-keto ester (**4.21**) (35mg, 0.068mmol, 1equiv) and butylamine (28μL, 0.27mmol, 4equiv), dissolved in toluene (1mL), at 0 °C. The solution, which turned orange-brown upon addition of $TiCl_4$, was allowed to warm to 20 °C and was then refluxed for 18h. The solvent was evaporated and purification by preparative tlc on silica, eluting with 80% petroleum ether/20% ethyl acetate yielded enamino ester (**4.07**) as a colourless oil (2mg, 6%): ^{1}H NMR ($CDCl_3$) δ 0.90 (t, J=6.3Hz, $CH_2CH_2CH_3$), 1.28 (t, J=7.1Hz, OCH_2CH_3), 1.30 (m, 4H, $NCH_2CH_2CH_2$), 2.98 (AB_q, J_{AB}=13.0Hz, 1H, $C3CH_aPh$), 3.03 (AB_q, J_{AB}=13.0Hz, 1H, $C3CH_bPh$), 3.55 (m, 3H , **(H-4)**$_a$ and NCH_2), 3.80 (AB_q, J_{AB}=18.8Hz, 1H, **(H-4)**$_b$), 4.15 (m, OCH_2CH_3), 4.98 (s, =C**H**), 5.05 (AB_q, J_{AB}=12.0Hz, 1H, OCH_aPh), 5.08 (AB_q, J_{AB}=12.0Hz, 1H, OCH_bPh), 5.27 (s, N**H**), 7.15 (m, **(Ph)**$_2$), 7.26 (m, **(Ph)**$_8$); HRMS (FAB, M+1) Found 465.2391 (Calcd for $C_{27}H_{30}N_2O_5$ 465.2389).

*Preparation of (3S) (E)-3-benzyl-3-benzyloxycarbonylamino-1-ethoxycarbonylmethyl-5-ethoxycarbonylmethylidene-2-pyrrolidinone (**4.02**):*

As above for the preparation of enamino ester (**4.07**), but with $TiCl_4$ (4μL, 0.037mmol, 0.5equiv) and glycine ethylester[E.07] (68mg, 0.66mmol, 10equiv) in a mixture of ether (1mL) and toluene (1mL). Purification by preparative tlc on silica, eluting with 98% CH_2Cl_2/2% ethyl acetate yielded enamino ester (**4.02**) as a pale yellow oil (4mg, 12%): ^{1}H NMR ($CDCl_3$) as given earlier (insertion method); HRMS (M) Found 494.2044 (Calcd for $C_{27}H_{30}N_2O_7$ 494.2053).

SECTION E.4.5

α-CHYMOTRYPSIN ASSAY

In the wells of a microtitre plate, 50mM Tris.HCl buffer, pH 7.6, (125μL) and 9 unit/mL α-chymotrypsin (Sigma, ex Porcine pancreas) solution in Tris. HCl buffer, pH 7.6, (50μL) were pre-incubated with either CH_3CN (25μL) or $2x10^{-4}$ mg/mL and $2x10^{-1}$ mg/mL CH_3CN test solutions of enamino esters and enollactones (**2.71, 3.04, 4.01, 4.02, 4.03, 4.06, 4.42, 4.43**) (25μL). After 30min at 37 °C, 1 mg/mL N-succinyl L-phenylalanine 4-nitro anilide in 500mM Tris.HCl buffer, pH 7.6, (100μL) was added and the optical density of the solutions was measured at 405nm. The solutions were incubated at 37 °C for 60min and then the optical density was again measured. Each sample was assayed in triplicate.

Samples blanks in which 50mM Tris HCl buffer, pH 7.6, replaced α-chymotrypsin were run concurrently.

Average absorbances were used to calculate the % inhibition (TABLE E.06).

TABLE E.06: Results of Chymotrypsin Assay

Compd	concn* (mmol/L)	% Inhibition	concn▼ (mmol/L)	% Inhibition
2.71	$7.8x10^{-4}$	0	$7.8x10^{-1}$	5
3.04	$4.9x10^{-4}$	0	$4.9x10^{-1}$	25
4.01	$3.5x10^{-4}$	20	$3.5x10^{-1}$	40
4.02	$4.0x10^{-4}$	0	$4.0x10^{-1}$	40
4.03	$3.6x10^{-4}$	5	$3.6x10^{-1}$	40
4.06	$3.9x10^{-4}$	0	$3.9x10^{-1}$	50
4.42E	$4.5x10^{-4}$	15	$4.5x10^{-1}$	40
4.42Z	$4.5x10^{-4}$	5	$4.5x10^{-1}$	35
4.43	$4.9x10^{-4}$	10	$4.9x10^{-1}$	25
	* all $2x10^{-4}$ mg/mL		▼ all $2x10^{-1}$ mg/mL	

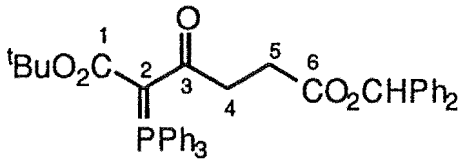
SECTION E.5

CHAPTER 5 EXPERIMENTAL

SECTION E.5.1

PREPARATION OF KETO ACID AND KETO ESTER PHOSPHORANES

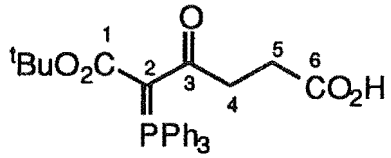
*Preparation of 6-Diphenylmethyl 1-(tert-butyl) 3-oxo-2-triphenylphosphoranylidenehexandioate (**5.04**):*



METHOD A: A solution of monobenzhydryl succinate[E.26] (**5.07**) (100mg, 0.34mmol, 1equiv) and a catalytic amount of DMF, in benzene (10mL), was cooled to 0 °C and freshly distilled oxalyl chloride (160μL, 1.83mmol, 5equiv) was slowly added. After 30min at 0 °C, the solution was allowed to warm to 20 °C and the solvent was evaporated. More benzene (5mL) was added and evaporated (repeated 3 times). The acid chloride (**5.08**) thus obtained was redissolved in benzene (10mL) and cooled to 0 °C. $Ph_3P{=}CHCO_2{}^tBu$[E.27] (256mg, 0.68mmol, 2equiv) was added and the mixture was stirred at 20 °C for 16h, filtered, and the solvent was evaporated. Purification by radial chromatography using a 2mm silica gel chromatotron plate, eluting with 90% $CHCl_3$/10% ethyl acetate yielded benzhydryl phosphorane (**5.04**) as an oil (198mg, 91%), which crystallized on standing: mp 163-164 °C (ether, colourless crystals); IR (nujol) 1740, 1760 and 1550cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.06 (s, C(C**H**$_3$)$_3$), 2.69 (t, J=7.2Hz, (**H-5**)$_2$), 3.27 (t, J=7.2Hz, (**H-4**)$_2$), 6.82 (s, C**H**Ph$_2$), 7.20-7.36 (m, (**Ph**)$_{10}$), 7.37-7.50 (m, (**Ph**)$_9$), 7.61-7.69 (m, (**Ph**)$_6$); ^{13}C NMR ($CDCl_3$) δ 28.16, 29.80, 35.21 (d, J=7.6Hz), 70.52 (d, J=109.5Hz), 76.38, 78.48, 127.05 (d, J=93.6Hz), 127.10, 127.52, 128.41 (d, J=12.7Hz), 131.36 (d, J=2.9Hz), 132.96 (d, J=9.7Hz), 140.61, 167.27 (d, J=13.7Hz), 172.84, 194.60 (d, J=4.3Hz); ^{31}P NMR ($CDCl_3$) δ 17.7; HRMS (FAB, M+1) Found 643.2610 (Calcd for $C_{41}H_{40}O_5P$ 643.2613). Anal. Calcd for $C_{41}H_{39}O_5P$: C 76.62; H 6.12. Found: C 76.50; H 6.20.

METHOD B: As for Method A except that acid chloride (**5.08**) was treated with $Ph_3P{=}CHCO_2{}^tBu$[E.27] (1equiv) and $^iPr_2NEt$ (1equiv): Yield after chromatography 27%; ^1H NMR ($CDCl_3$) as given above.

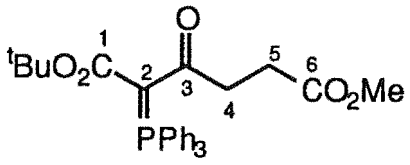
*Preparation of 1-(tert-Butyl) 3-oxo-2-triphenylphosphoranylidenehexandioate (**5.05**):*



METHOD A: TFA (1.0mL, 13.0mmol, 170equiv) was added to a solution of benzhydryl ester (**5.04**) (50mg, 0.078mmol, 1equiv) in CH_2Cl_2 (2mL), at 0 °C. After stirring for 5min, the solution was diluted with CH_2Cl_2 (13mL), washed with water (4x 15mL), dried ($MgSO_4$) and the solvent was evaporated to yield keto acid phosphorane (**5.05**) as a colourless oil (quant), which crystallized on standing at 4 °C: IR (KBr) 3425, 1740, 1680 and 1540cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.05 (s, C(**CH_3**)$_3$), 2.55 (t, J=5.8Hz, (**H-5**)$_2$), 3.32 (t, J=5.8Hz, (**H-4**)$_2$), 7.34-7.71 (m, (**Ph**)$_3$); ^{13}C NMR ($CDCl_3$) δ 27.95, 32.00, 33.63 (d, J=7.3Hz), 73.81 (d, J=107.6Hz), 79.90, 125.51 (d, J=93.7Hz), 128.78 (d, J=12.5Hz), 132.11 (d, J=3.0Hz), 132.98 (d, J=10.1Hz), 166.60 (d, J=12.4Hz), 175.23, 196.52 (d, J=3.7Hz); ^{31}P NMR ($CDCl_3$) δ 17.3; HRMS (FAB, M+1) Found 477.1834 (Calcd for $C_{28}H_{30}O_5P$ 477.1831).

METHOD B: $Ph_3P{=}CHCO_2{}^tBu$[E.27] (100mg, 0.27mmol, 1equiv) was added to a stirred solution of succinic anhydride (27mg, 0.27mmol, 1equiv) in CH_2Cl_2 (5mL). After 2h the solution was poured onto cold petroleum ether. Crystallization did not occur, hence the solvent was evaporated to yield a colourless solid which contained, by ^1H NMR, 46% desired keto acid phosphorane (**5.05**) (^1H NMR ($CDCl_3$) as given above), 27% recovered $Ph_3P{=}CHCO_2{}^tBu$ and 27% recovered succinic anhydride.

Preparation of 6-Methyl 1-(tert-butyl) 3-oxo-2-triphenylphosphoranylidenehexandioate **(5.06):**



METHOD A: Acid phosphorane (**5.05**) (Sample from Method B above) was dissolved in THF (2mL), cooled to 0 °C and treated with freshly distilled CH_2N_2 in ether. Excess CH_2N_2 was allowed to evaporate at 20 °C over 16h and the residue was purified by radial chromatography using a 1mm silica gel chromatotron plate, eluting with 80% $CHCl_3$/20% ethyl acetate to yield phosphorane (**5.06**) as an oil (45mg, 41% from succinic anhydride): mp 165-167 °C (ether, colourless crystals); IR (nujol) 1740, 1670 and 1550cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.06 (s, C(**CH$_3$**)$_3$), 2.55 (t, J=7.0Hz, (**H-5**)$_2$), 3.23 (t, J=7.0Hz, (**H-4**)$_2$), 3.57 (s, OC**H$_3$**), 7.39-7.50 (m, (**Ph**)$_9$), 7.64-7.71 (m, (**Ph**)$_6$); ^{13}C NMR ($CDCl_3$) δ 28.10, 29.28, 35.38 (d, J=7.3Hz), 51.30, 70.50 (d, J=109.8Hz), 78.49, 127.15 (d, J=93.1Hz), 128.46 (d, J=12.1Hz), 131.38 (d, J=3.0Hz), 133.00 (d, J=9.8Hz), 167.35 (d, J=13.8Hz), 174.39, 194.90 (d, J=4.5Hz); ^{31}P NMR ($CDCl_3$) δ 17.2; HRMS (FAB, M+1) Found 491.1989 (Calcd for $C_{29}H_{32}O_5P$ 491.1988). Anal. Calcd for $C_{29}H_{31}O_5P$: C 71.01; H 6.17. Found: C 71.22; H 6.35.

METHOD B: $MeO_2C(CH_2)_2COCl$[E.15] (176mg, 1.17mmol, 1equiv) was dissolved in benzene (10mL) and cooled to 0 °C. $Ph_3P{=}CHCO_2{}^tBu$[E.27] (881mg, 2.34mmol, 2equiv) was added and the mixture was stirred at 20 °C for 16h, filtered, and the solvent was evaporated. Purification by radial chromatography using a 2mm silica gel chromatotron plate, eluting with 90% $CHCl_3$/10% ethyl acetate yielded phosphorane (**5.06**) as a white solid (292mg, 51%): ^{1}H NMR ($CDCl_3$) as given above.

SECTION E.5.2

MASS SPECTROMETRY OF KETO ACID AND KETO ESTER PHOSPHORANES

Positive ion FAB spectra were obtained using a Kratos MS80RFA mass spectrometer operated at 4KV, with a resolution of 1000, scanning at 3 seconds per decade and equipped with an Ion Tech ZN11NF saddle field FAB gun, operated at 8kV, 2mA ion current with xenon (Xe) as the reagent gas. High resolution results were obtained with a resolution of 7500 using polyethyleneglycol (PEG) and modified PEG's as reference compounds[E.28] for peak matching. The sample (**4.15, 4.26, 4.40-4.41, 5.04-5.06**) (5mL of 10 mg/mL solution in $CHCl_3$) was deposited onto a copper target containing matrix (5mL). The ions observed and their relative abundances are shown in TABLE E.07. The matrices used were nitrobenzyl alcohol and a 1 : 5 mixture of dithioerythritol and dithiothreitol ("magic bullet").

TABLE E.07:. Relative abundance of positive ions observed for the keto acid and keto ester phosphoranes (**4.15, 4.26, 4.40-4.41, 5.04-5.06**) in FAB.

No	*m/z* (relative intensity, %)
4.15	776 (38), 730 (35), 390 (35), 375 (80), 303 (100), 279 (32), 262 (51), 201 (51), 183 (80)[a]
4.26	746 (81), 700 (56), 390 (65), 375 (89), 303 (100), 279 (26), 262 (63), 201 (38), 183 (56), 165 (45)[a]
4.40	688 (45), 642 (20), 612 (21), 508 (31), 390 (41), 375 (71), 349 (25), 303 (100), 279 (39), 262 (53), 225 (20), 201 (38), 183 (65)[a]
4.41	702 (86), 656 (22), 390 (64), 375 (71), 303 (100), 279 (21), 262 (43), 201 (22), 183 (38)[a]
5.04	643 (10), 569 (8), 403 (15), 347 (30), 303 (30), 279 (7), 262 (8), 201 (34), 183 (15), 167 (100), 152 (12)[b]
5.05	477 (41), 403 (54), 377 (58), 347 (45), 321 (99), 303 (82), 279 (29), 262 (24), 201 (21), 183 (58), 152 (100)[b]
5.06	491 (41), 417 (57), 403 (11), 347 (56), 303 (100), 279 (26), 262 (15), 201 (18), 183 (32), 152 (16)[b]
	[a] Spectra run in nitrobenzyl alcohol [b] Spectra run in "magic bullet"

SECTION E.6

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